

Soil invertebrates in an Ultisol soybean agroecosystem amended with urea-enriched cogongrass biochar prepared with *Sargassum* extract

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Abstract. Kilowasid LMH, Al'adlin, Alam S, Rakian TC, Rahni NM, Adawiyah R. 2026. Soil invertebrates in an Ultisol soybean agroecosystem amended with urea-enriched cogongrass biochar prepared with *Sargassum* extract. *Biodiversitas* 27 (1): d270116. <https://doi.org/10.13057/biodiv/d270116>. Soil invertebrates are widely used as bioindicators to assess changes in ecological functioning under different management practices. However, information on the influence of enriched biochar amendments on the community of invertebrates in tropical Ultisol remains limited, particularly in the context of reducing dependency on synthetic fertilizer through organic-based management. Therefore, this study aimed to evaluate the interactive effects of cogongrass (*Imperata cylindrica*) biochar doses and urea concentrations dissolved in *Sargassum* seaweed extract on the abundance and diversity of soil invertebrates in an Ultisol-based soybean agroecosystem. A split-plot experiment was conducted using three biochar doses (0, 5, and 10 t ha⁻¹) and four urea solution concentrations (0, 5, 10, and 20%) as main plots and subplots, respectively. Soil-surface invertebrates were collected using pitfall traps, while soil-living invertebrates were sampled through hand-sorting and monolith methods, and identified at the family level. The results showed that biochar and urea amendments significantly restructured the community of soil invertebrates, with contrasting responses between soil-surface and soil-living groups. Biochar dose primarily influenced surface-active invertebrates, and urea concentration affected specific soil-living taxa, with interaction effects observed for selected functional groups. Higher biochar doses increased community diversity, as reflected by higher Shannon-Wiener and lower Simpson indices, while moderate urea concentrations supported the highest taxon richness. Optimized biochar-urea combinations enhanced the abundance of predatory invertebrates, particularly ants (Formicidae), without reducing overall taxonomic diversity. These findings show that urea-enriched cogongrass biochar prepared with *Sargassum* extract can improve soil ecological functioning through beneficial restructuring of the community of invertebrates, supporting its potential role in sustainable nutrient management and biodiversity-based restoration of degraded tropical Ultisol agroecosystem.

Keywords: Community composition, functional groups, nutrient management, soil fauna, tropical agroecosystem

INTRODUCTION

Soil invertebrates are crucial in regulating ecological processes that underpin productivity and stability in agroecosystems. Through their role in organic matter decomposition, nutrient mineralization, soil aggregation, and trophic interactions, the invertebrates directly influence soil fertility and crop performance (Kopittke et al. 2019; Bravo et al. 2024). In legume-based systems, such as soybeans (*Glycine max* (L.) Merr.), surface and soil-living invertebrates contribute to nutrient cycling and biomass production, making community attributes sensitive indicators of soil ecological functioning under different management practices (Anggraini et al. 2021; Sial et al. 2022; Shehzad et al. 2024).

In tropical agricultural systems, including in Indonesia, soil invertebrate communities respond rapidly to changes in soil resources and habitat conditions induced by land management (Menta and Remelli 2020). Evidence from Indonesian Ultisol shows that organic matter inputs significantly modify the abundance and diversity of mesofauna, reflecting shifts in carbon availability and

microhabitat quality (Barus et al. 2021). Similarly, studies in Indonesian soybean agroecosystems suggest that fertilization regimes influence arthropod community attributes, with organic or integrated nutrient inputs generally supporting higher abundance and diversity than purely inorganic fertilization (Hasibuan et al. 2022). These results confirm the responsiveness of soil invertebrates to nutrient and organic amendments and can serve as ecological indicators in tropical agroecosystems.

Soybean is a strategic crop in Indonesia as the primary raw material for widely consumed foods such as tofu and tempeh, and improving its productivity is essential for national food security (Rozi et al. 2025). However, the cultivation is largely constrained by the dominance of Ultisol in dryland agricultural areas. Ultisol is characterized by low organic matter content, strong soil acidity, and limited nutrient availability, particularly nitrogen, which restricts root development, biological activity, and crop yield (Purwanto et al. 2021; Pulunggono et al. 2022). Rehabilitation efforts on the soil commonly focus on increasing organic carbon and nutrient availability to improve soil structure, water retention, and biological activity (de

Corato 2020). These interventions may also alter habitat conditions and resource distribution, thereby restructuring soil invertebrate communities that regulate key ecosystem processes (Delgado-Baquerizo et al. 2018). Despite the correlation, soil fauna responses to amendments in Ultisol-based soybean systems remain poorly documented, particularly in tropical environments (Nsengimana et al. 2021).

Biochar has been promoted as a soil amendment to improve physicochemical properties through carbon stabilization, pH buffering, and enhanced cation exchange capacity, making it relevant for acidic and nutrient-poor soil, including Ultisol (Liu et al. 2025). However, the effects of biochar on soil biota are inconsistent and vary with feedstock type, pyrolysis conditions, and application rate (Wang et al. 2021). Several studies have reported that high application rate or chemically reactive biochar formulations can negatively affect soil fauna, underscoring the need for formulation-specific evaluation (da Costa et al. 2024; Li et al. 2024). To address these limitations, nitrogen-enriched biochar has been developed to improve nutrient availability while maintaining biological functioning (Jia et al. 2021).

Cogongrass biochar enriched with urea dissolved in *Sargassum* seaweed extract represents an amendment strategy tailored to degraded tropical soil. As an abundant weed in Indonesia, cogongrass is a locally available and sustainable biochar feedstock. Enrichment with urea aims to improve nitrogen retention and reduce N losses, while *Sargassum* extract provides bioactive compounds that may enhance nutrient efficiency and stimulate biological activity (Godlewska et al. 2016; Jia et al. 2021). Previous studies reported that the enriched biochar improves surface chemical properties, nutrient-binding capacity, soil water retention, arbuscular mycorrhizal symbiosis, and soybean nodulation (Kilowasid et al. 2024, 2025). However, existing (Nsengimana et al. 2021; Wang et al. 2021) studies on urea-enriched cogongrass prepared with *Sargassum* extract have largely focused on soil physicochemical properties and microbial or symbiotic responses, with limited attention to invertebrate communities. Most biochar-fauna studies have been conducted in temperate systems or using simplified biochar formulations, leaving uncertainty regarding the influence of enriched biochar and nitrogen availability interaction on soil invertebrates in tropical Ultisol.

This study aimed to evaluate the independent and interactive effects of cogongrass biochar doses and urea concentrations dissolved in *Sargassum* extract on the abundance, richness, and diversity of soil invertebrates in an Ultisol-based soybean agroecosystem. By examining soil-surface and soil-living invertebrate communities, the results provide insight into the influence of urea-enriched cogongrass biochar on ecological functioning, particularly the biological processes mediated by soil invertebrates. It also supports sustainable soil management strategies for degraded tropical soil.

MATERIALS AND METHODS

Experimental site

The experiment was conducted within a cogongrass dominated-Ultisol area in Tanea District, South Konawe Regency, Southeast Sulawesi, Indonesia (4°3'4.99" S; 122°52'17.64" E; 46 masl). The experimental site covered approximately 0.75 ha and was previously cultivated with maize (*Zea mays* L.). The field had been abandoned since 2018 and was left unmanaged, allowing natural regrowth dominated by cogongrass.

In 2024, a 0.5 ha portion of this abandoned field was prepared and established as the experimental area. The site was classified as Ultisol according to U.S. Department of Agriculture (USDA) Soil Taxonomy based on soil morphological and chemical characteristics (Kilowasid et al. 2025). The area exhibited flat topography (0-3% slope) and relatively homogeneous vegetation prior to land preparation. Permission to use the land for research purposes was obtained from the local farmer, landowner and village authorities. The experimental site is not located within a protected or restricted area.

Climatic data were obtained from the Southeast Sulawesi Climatology Station, Meteorology, Climatology, and Geophysics Agency (BMKG). During the experimental period from June to December 2024, the site received an average monthly rainfall of 134.37 mm, with a mean air temperature of 26.86°C and relative humidity of 85.29%. These represent typical humid tropical conditions for rainfed soybean cultivation in the region.

Soil properties in Table 1 represent baseline (pre-treatment) conditions measured before biochar application from composite samples collected across the experimental field. Physical and chemical properties were determined using standard procedures at the Soil and Fertilizer Instrument Standard Testing Laboratory, Bogor, Indonesia.

Experimental design

The experiment was arranged in a split-plot randomized complete block design with three replications. Cogongrass biochar dose (D) was assigned as the main-plot factor with three levels at 0 t ha⁻¹ (D0), 5 t ha⁻¹ (D5), and 10 t ha⁻¹ (D10). Urea concentration dissolved in *Sargassum* extract (C) was assigned as the subplot factor with four levels at 0% (C0), 5% (C5), 10% (C10), and 20% (C20). Urea fertilizer (46%N) was used as a nitrogen source for biochar enrichment. These concentrations were selected based on previous studies on nitrogen-enriched biochar and liquid seaweed extract (Kilowasid et al. 2025). Blocks, main plots, and subplots were spatially arranged following a standard split-plot field layout, as detailed in Figure 1, to minimize edge effects and ensure independent experimental units. In total, 36 plots (3 blocks × 3 biochar doses × 4 urea concentrations) were established, with each serving as the experimental unit for soil invertebrate community assessment.

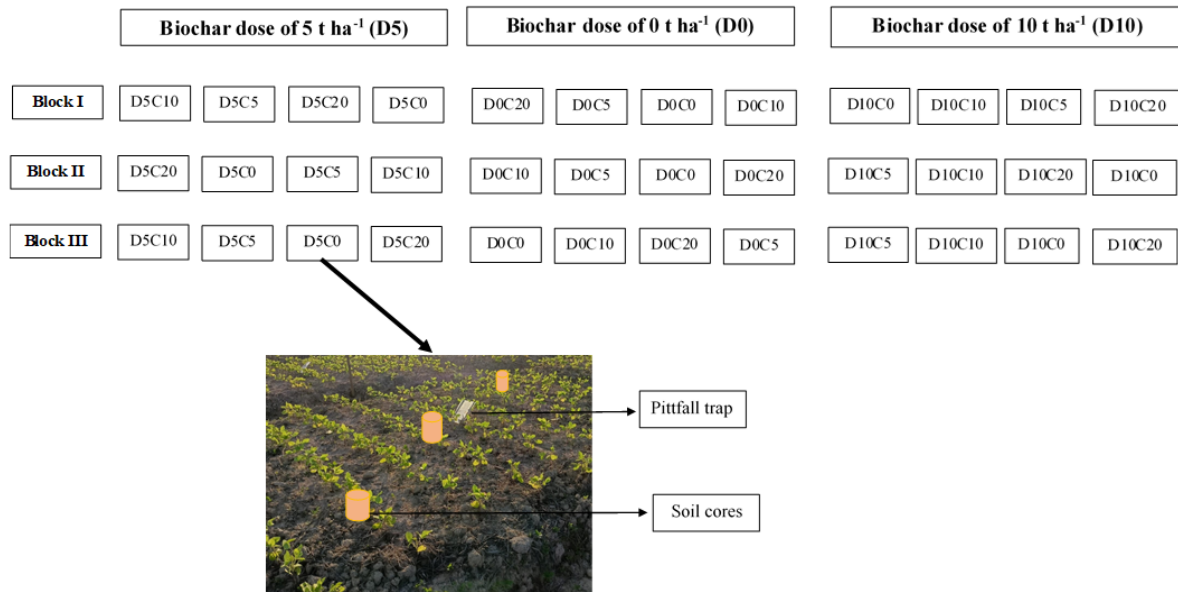


Figure 1. Schematic representation of the split-plot layout in the field, showing three experimental blocks, cogongrass biochar doses (0, 5, and 10 t ha⁻¹; D0, D5, D10) as main plots, and urea concentrations dissolved in *Sargassum* extract (C0, C5, C10, C20) as subplots. Symbols indicate pitfall trap placement (one per plot) and soil core sampling points (three per plot: the plot center and two diagonally opposite corners). The bottom panel presents a representative field view illustrating the pitfall trap and soil core positions. Not to scale

Table 1. Physical and chemical properties of pre-treatment of the Ultisol at the experimental site

Soil property	Value	Method
Physical properties		
Bulk density (g cm ⁻³)	1.10 (1.03-1.22)	Gravimetric
Total porosity (%)	58.62 (54.13-61.05)	Gravimetric
Sand (%)	13.50 (7-19)	Pipette method
Silt (%)	52.67 (44-58)	Pipette method
Clay (%)	33.83 (29-41)	Pipette method
Chemical properties		
pH (H ₂ O, 1:5)	5.55 (5.4-5.8)	Electrometric
Electrical conductivity (%)	0.04 (0.01-0.082)	Conductivity meter
Organic C (%)	1.53 (1.36-1.73)	Walkley-Black
Total N	0.21 (0.18-0.23)	Kjeldahl
C:N ratio	7.83 (7-8)	Calculation
P ₂ O ₅ (mg 100 g ⁻¹)	20.67 (18-28)	25% HCl extraction
K ₂ O (mg 100 g ⁻¹)	15.83 (12-18)	25% HCl extraction
Exchangeable Al ³⁺ (cmol kg ⁻¹)	1.87 (0.04-4.03)	1 N KCl extraction
Exchangeable H ⁺ (cmol kg ⁻¹)	1.85 (0.80-2.89)	1 N KCl extraction

Note: Values represent means (minimum-maximum) from five initial soil samples collected prior to treatment application

Preparation and application of enriched cogongrass biochar

Cogongrass straw was sun-dried and converted into biochar through slow pyrolysis at 350-440°C for 15-30 min

using a drum-type pyrolyzer, then ground and sieved to <3 mm/opening (Yang and Lu 2021; Kilowasid et al. 2025). *Sargassum* seaweed extract was prepared by drying and milling biomass, followed by maceration in warm water (40°C) for 24 h and filtration to obtain a stock extract (Godlewska et al. 2016). Furthermore, urea fertilizer (46% N) was dissolved in diluted extract to achieve the target concentrations. Biochar was enriched by mixing sieved biochar with the urea-*Sargassum* solution at a 1:1 (w/v) ratio and air-dried to its initial dry mass (Kilowasid et al. 2024). Urea-*Sargassum* solution was applied only during the biochar-enrichment step prior to field application. It was important to acknowledge that no additional urea solution was applied after plot establishment.

Enriched biochar was applied uniformly to each plot, 3 × 4 m, and incorporated into the 0-15 cm soil layer, followed by a 20-day incubation period to allow nutrient stabilization (Kilowasid et al. 2025). Subsequently, soybean (*G. max*) cultivar Argomulyo was planted at 40 × 20 cm spacing. During dry periods, supplemental irrigation was applied, while weed and pest control were conducted manually.

Soil invertebrates sampling and identification

Each plot contained one pitfall trap placed at the center between soybean rows. Soil-living invertebrates were sampled at physiological maturity stage of soybean (R8, full maturity), corresponding to the end of the crop growth cycle, using three soil cores per plot. Accordingly, pitfall-trap observations comprised 36 traps × 5 sampling occasions, while soil-core sampling included 108 cores (36 plots × 3 cores per plot). Surface-active soil invertebrates were sampled using pitfall traps constructed from plastic containers (8 cm diameter × 10 cm height). The traps were

partially filled with 70% ethanol supplemented with a few drops of unscented detergent as a killing and preservative solution used to reduce surface tension and improve specimen preservation (Souza et al. 2012). The deployment lasted for 48 h at five soybean growth stages, namely V2 (second trifoliolate), R1 (beginning bloom), R3 (beginning pod), R5 (beginning seed), and R8 (physiological maturity), to capture temporal variation in surface-active invertebrate communities.

Although Diptera and non-ant Hymenoptera are highly mobile taxa, individuals captured in pitfall traps represent surface-active assemblages associated with the soil-litter interface rather than strictly soil-dwelling fauna. Therefore, this present study distinguishes between soil-living (endogeic taxa) and surface-active invertebrates (epigeic taxa sensu Menta and Remelli 2020). Larval stages were not separated from adults during pitfall trapping. As a result, ecological interpretations for Diptera and non-ant Hymenoptera are restricted to the surface activity and functional association with soil-derived resources rather than direct soil residency.

At physiological maturity (R8), soil-living invertebrates were collected using three soil cores per plot (15 cm diameter × 10 cm depth), taken from the center and two diagonally opposite corners to capture within-plot spatial heterogeneity. Invertebrates were extracted by hand-sorting for 20 person-minutes per sample on a light-colored tray (Damayanti et al. 2023). Collected specimens were immediately preserved in 70% ethanol, acting as an antiseptic to prevent microbial degradation. However, earthworms were preserved in 4% formalin, serving as an antiseptic to inhibit microbial growth and tissue decomposition (Hamamoto et al. 2024).

All specimens were identified at least to the order level, and major taxa relevant to agroecosystem functioning were further resolved to the family level when feasible (e.g., Formicidae and Glossoscolecidae), using standard taxonomic keys (Borror et al. 1989; Dindal 1990; James 2004). This method was adopted to ensure consistent identification across repeated sampling occasions and large sample volumes, while retaining sufficient ecological resolution for detecting treatment-related shifts in community structure (Decaëns et al. 2006; Menta and Remelli 2020). Accordingly, diversity metrics reported in this study represent taxon-level (order/family) rather than species-level diversity. In highly diverse tropical agroecosystems, taxon-level (order/family) is widely accepted as a robust surrogate for species-level when the objective is to assess community-level and functional responses to management interventions.

This is attributed to the ability to minimize identification bias while preserving meaningful ecological interpretation of soil ecological functioning.

Data analysis

Pitfall-trap data collected across growth stages were averaged per plot to obtain seasonal estimates of abundance and diversity for statistical analysis. Data from three cores per plot were treated as subsamples and averaged, producing one value per plot for soil-living invertebrates.

The effects of biochar dose, urea concentration, and the interaction on soil invertebrate relative abundance, taxon richness, and diversity indices were analyzed using analysis of variance (ANOVA) in a split-plot randomized block design at $p < 0.05$. The response variables were calculated per plot ($n = 36$ plots), generating one value per plot for each metric used in the split-plot ANOVA.

Prior to ANOVA, residuals were tested for normality and homogeneity of variance using the Shapiro-Wilk and Levene's test. When necessary, data were $\log(x + 1)$ or square-root transformed to meet ANOVA assumptions. Post hoc comparisons were performed using Tukey's honestly significant difference (HSD) test at $p < 0.05$, which inherently controls the family-wise error rate for multiple comparisons (Steel and Torrie 1981). All statistical analyses were performed by IBM SPSS Statistics version 24.0.

Community metrics included taxon richness (number of orders/families, S), Shannon-Wiener diversity index (H'), Simpson dominance index (D), and Pielou's evenness (E). The following equations were used to calculate the metrics (Kurniawan et al. 2023; Rahmawati et al. 2024):

$$H' = - \sum_{i=1}^n (p_i) (\ln p_i)$$

$$D = \sum (p_i)^2$$

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

In the equation, p_i denotes the proportional abundance ($\frac{n_i}{N}$), n_i is the number of individuals belonging to the i -th taxon, N is the total number of individuals across all taxa, \ln is the natural logarithm, and S represents the total number of taxa. Since identification was conducted at the order level (and selected families), Shannon-Wiener, Simpson, and evenness indices were computed and interpreted as taxon-level diversity indices rather than species diversity.

RESULTS AND DISCUSSION

Surface-active invertebrates (pitfall traps)

Analysis of variance showed that cogon grass biochar dose significantly affected the relative abundance of Diptera ($F_{2,4} = 11.323$, $p = 0.023$) and Formicidae ($F_{2,4} = 9.107$, $p = 0.032$), while urea solution concentration significantly influenced Hymenoptera abundance ($F_{3,18} = 3.483$, $p = 0.037$). Relative abundance (%) was calculated as the number of individuals of a given taxon divided by the total number captured per plot, multiplied by 100, and values were averaged across the three experimental blocks ($n = 3$). A significant interaction between biochar dose and urea concentration was detected only for Orthoptera ($F_{6,18} = 2.902$, $p = 0.037$). Based on observation, no other surface-active taxa showed significant interaction effects as detailed in Table 2.

Post hoc comparisons showed that Diptera abundance peaked at the moderate biochar dose (5 t ha^{-1}) and was

significantly higher than at 10 t ha⁻¹, while the control (0 t ha⁻¹) did not differ significantly from either treatment (Tukey's HSD, $p > 0.05$), as detailed in Table 3. Formicidae abundance was highest at 10 t ha⁻¹, differing significantly from the control and not from the intermediate dose. Hymenoptera abundance was lowest at 10% urea concentration and differed significantly from 0, 5, and 20%. However, the three concentrations did not differ from each other.

A significant biochar \times urea interaction was detected only for Orthoptera ($p = 0.037$). This suggests that Orthoptera

abundance was dependent on specific treatment combinations rather than on individual main effects. The abundance was highest under combinations of low-to-moderate biochar doses and moderate urea concentrations, as detailed in Figure 2, but no consistent pattern was observed across single-factor gradients.

At the community level, taxon-level richness and diversity indices (Shannon-Wiener, Simpson, and Pielou's) of surface-active assemblages were not significantly affected by biochar dose, urea concentration, or the interaction (split-plot ANOVA, $p > 0.05$ for all indices; Table 2).

Table 2. Split-plot ANOVA effects of cogongrass biochar dose (D), urea solution concentration (C), and the interaction (D \times C) on surface-active invertebrate taxa and community indices

Variable	Dose of biochar (D)			Concentration of urea (C)			Interaction (D \times C)		
	MS	F	Sig.	MS	F	Sig.	MS	F	Sig.
Taxon									
Arachnida	0.147	0.860	0.489	0.046	0.318	0.812	0.111	0.762	0.609
Amphipoda	0.072	3.045	0.157	0.012	0.692	0.569	0.012	0.692	0.659
Araneae	0.061	0.370	0.712	0.083	0.415	0.744	0.436	2.181	0.093
Blattodea	0.020	0.908	0.473	0.027	0.429	0.735	0.053	0.844	0.553
Coleoptera	1.020	0.945	0.461	0.212	0.286	0.835	0.270	0.365	0.892
Dermaptera	0.085	2.890	0.167	0.018	0.499	0.687	0.016	0.436	0.845
Diptera	1.928	11.323	0.023	0.305	0.615	0.614	0.536	1.079	0.411
Formicidae	2.126	9.107	0.032	0.053	0.088	0.965	0.246	0.411	0.862
Hemiptera	4.094	0.605	0.589	0.153	0.071	0.974	4.307	2.017	0.116
Hymenoptera	0.202	0.405	0.691	0.691	3.483	0.037	0.409	2.061	0.110
Isopoda	0.216	3.498	0.132	0.099	0.974	0.427	0.128	1.258	0.324
Lepidoptera	0.028	0.906	0.474	0.096	2.570	0.086	0.019	0.512	0.791
Orthoptera	0.106	2.748	0.177	0.341	1.417	0.270	0.698	2.902	0.037
Thysanoptera	0.085	0.464	0.659	0.137	1.773	0.188	0.045	0.588	0.736
Diversity index									
Richness (R)	1.000	0.300	0.756	1.657	1.218	0.332	0.630	0.463	0.827
Shannon-Wiener (H')	0.006	0.256	0.786	0.007	0.569	0.642	0.011	0.896	0.519
Simpson (D)	0.000	0.385	0.703	0.001	0.882	0.469	0.001	0.782	0.595
Evenness (E)	0.000	0.044	0.958	0.004	1.701	0.202	0.002	0.718	0.640

Note: Values in bold in the **Sig.** column indicate statistical significance at $p < 0.05$. df for D = 2,4; C = 3,18; D \times C = 6,18. MS: mean square; Sig.: significance level

Table 3. Relative abundance of surface-soil invertebrate taxa affected by biochar dose or urea concentration

Treatment level	Relative abundance (%)		
	Diptera	Formicidae	Hymenoptera
Biochar dose (D)			
0 t/ha	6.28 \pm 0.70 ^{ab}	25.63 \pm 2.51 ^{ab}	-
5 t/ha	9.99 \pm 1.32 ^b	23.15 \pm 4.41 ^a	-
10 t/ha	5.67 \pm 1.29 ^a	31.46 \pm 3.61 ^b	-
Urea concentration (C)			
0%	-	-	4.34 \pm 1.11 ^b
5%	-	-	4.16 \pm 1.84 ^b
10%	-	-	1.95 \pm 0.60 ^a
20%	-	-	3.27 \pm 0.86 ^b

Note: Values are means \pm SD (n=3). - shows the taxon was present but did not show a significant main effect for the corresponding factor in the split-plot ANOVA and was therefore not subjected to post hoc comparison. Means within the same column followed by different superscript letters differ significantly (Tukey HSD, $p < 0.05$)

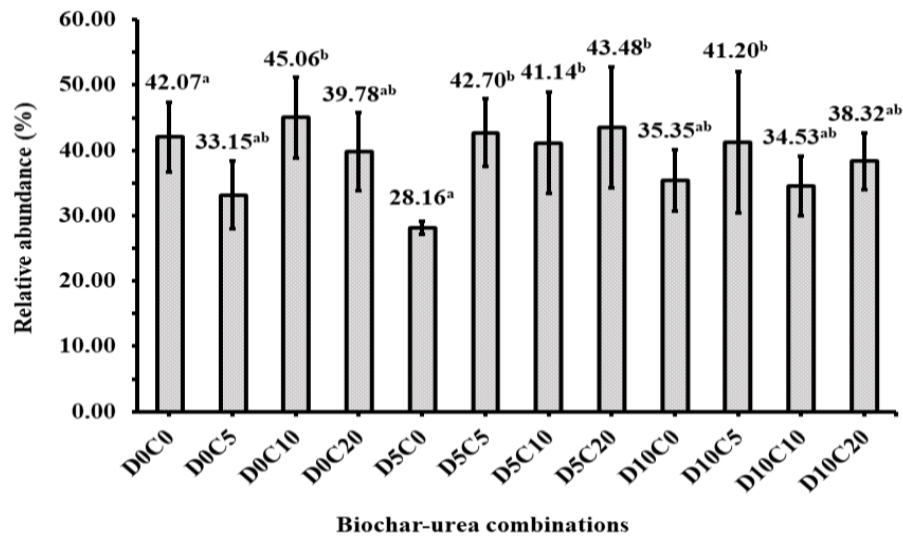


Figure 2. Relative abundance (%) of Orthoptera under combinations of cogongrass biochar dose (D0: 0 t ha⁻¹, D5: 5 t ha⁻¹, D10: 10 t ha⁻¹) and urea concentration dissolved in *Sargassum* extract (C0: 0%, C5: 5%, C10: 10%, C20: 20%). Bars represent mean \pm SD (n = 3). Different letters show significant differences among treatment combinations (Tukey HSD, p<0.05)

Table 4. Summary of significant ANOVA effects of cogongrass biochar dose (D), urea solution concentration (C), and the interaction (D×C) on living-soil invertebrate taxa

Taxon	Biochar dose (D)	Urea concentration (C)	Interaction (D×C)
Araneae	Yes (p = 0.005)	No	No
Formicidae	Yes (p = 0.032)	Yes (p = 0.048)	No
Glossoscolecidae	Yes (p = 0.043)	Yes (p = 0.029)	No

Note: “Yes” shows a statistically significant effect at p<0.05 based on split-plot ANOVA. Complete ANOVA results for all taxa, including non-significant responses, are provided in Table 5

Soil-living invertebrates

For soil-living invertebrates, biochar dose significantly affected the relative abundance of Araneae (p = 0.005), Formicidae (p = 0.032), and Glossoscolecidae (p = 0.043), while urea concentration significantly influenced Formicidae (p = 0.048) and Glossoscolecidae (p = 0.029). No significant biochar \times urea interaction was detected for any soil-living taxon, as detailed in Tables 4 and 5.

Post hoc analysis showed that Araneae abundance increased significantly at the highest biochar dose (10 t ha⁻¹), while Glossoscolecidae declined progressively with increasing biochar dose (Table 6). Formicidae abundance was highest at 10 t ha⁻¹ and increased with higher urea concentrations. Significant differences were detected only between the lowest and highest urea levels, as detailed in Table 6.

Community-level analysis showed that Shannon-Wiener diversity increased with higher biochar dose. Meanwhile, Simpson dominance featured an inverse pattern, with significant differences between the lowest and highest biochar doses (Figures 3.A-3.B). Taxon richness varied

significantly among urea concentrations, reaching the highest value at the moderate urea level (5%) (Figure 3; Table 5). Taxon richness varied significantly among urea concentrations, reaching the highest value at 5% as detailed in Figure 4.

Overall patterns of soil invertebrate community responses

Enriched cogongrass biochar application restructured soil invertebrate communities in the Ultisol-based soybean agroecosystem, as reflected by taxon-specific responses and taxon-level diversity patterns across surface-active (epigeic) and soil-living (endogeic) assemblages (Tables 2-3; Figures 2-4). Surface-active taxa were primarily regulated by biochar dose and biochar \times urea interactions, as detailed in Table 2; Figure 2, while soil-living taxa responded mainly to biochar dose and urea concentration independently, as detailed in Tables 4 and 6. Taxon-level diversity indices showed that higher biochar doses enhanced community diversity without reducing taxon-level richness, as detailed in Figures 3 and 4.

Table 5. Complete split-plot ANOVA results for living-soil invertebrate taxa and community indices

Variable	Dose of biochar (D)			Concentration of urea (C)			Interaction (D×C)		
	MS	F	Sig.	MS	F	Sig.	MS	F	Sig.
Taxon									
Araneae	2.803	25.801	0.005	0.044	0.137	0.937	0.044	0.136	0.990
Blattodea	0.030	1.000	0.444	0.030	1.000	0.433	0.030	1.000	0.415
Coleoptera	0.009	0.037	0.964	0.500	0.884	0.468	0.797	1.411	0.264
Dermaptera	0.137	0.807	0.508	0.396	1.791	0.185	0.520	2.352	0.075
Diptera	0.116	0.567	0.607	0.115	0.930	0.446	0.068	0.547	0.766
Formicidae	2.226	9.245	0.032	1.167	3.200	0.048	0.450	1.233	0.336
Glossoscolecidae	1.507	7.696	0.043	2.063	3.765	0.029	0.681	1.242	0.331
Hemiptera	0.025	1.000	0.444	0.025	1.000	0.415	0.025	1.000	0.455
Isopoda	0.019	1.000	0.444	0.019	1.000	0.415	0.019	1.000	0.455
Lepidoptera	0.056	1.000	0.444	0.216	1.979	0.153	0.056	0.511	0.792
Orthoptera	0.083	0.839	0.496	0.032	0.312	0.817	0.097	0.946	0.487
Thysanoptera	0.043	1.730	0.287	0.027	0.816	0.502	0.027	0.816	0.571
Diversity index									
Richness (R)	0.085	4.284	0.101	0.137	3.620	0.033	0.037	0.968	0.474
Shannon-Wiener (H')	0.045	8.008	0.040	0.021	2.605	0.084	0.009	1.123	0.388
Simpson (D)	0.015	12.722	0.018	0.004	1.579	0.229	0.003	1.194	0.354
Evenness (E)	0.022	4.055	0.109	0.011	1.540	0.238	0.010	1.507	0.232

Note: Values in bold in the Sig. column indicate statistical significance at $p < 0.05$. df for D = 2,4; C = 3,18; D×C = 6,18. MS: mean square; Sig.: significance level

Table 6. Relative abundance (%) of living-soil invertebrate taxa affected by biochar dose or urea concentration

Level of treatment factor	Relative abundance of soil invertebrate taxon		
	Araneae	Formicidae	Glossoscolecidae
Biochar dose			
0 t/ha	0.93±1.06 ^a	11.42±11.16 ^a	70.51±20.26 ^b
5 t/ha	0.30±0.52 ^a	30.04±8.47 ^a	41.50±9.07 ^{ab}
10 t/ha	17.76±9.26 ^b	41.15±16.87 ^b	17.79±9.20 ^a
Urea concentration			
0%	-	12.82±6.66 ^a	64.43±10.98 ^b
5%	-	20.55±5.42 ^{ab}	52.56±22.72 ^b
10%	-	33.83±18.45 ^{ab}	25.31±24.17 ^a
20%	-	42.96±13.65 ^b	30.78±4.78 ^{ab}

Note: Values are means ±SD (n = 3). - shows the taxon was present but did not show a significant main effect for the corresponding factor in the split-plot ANOVA and was therefore not subjected to post hoc comparison. Means within the same column followed by different superscript letters are significantly different (Tukey HSD, $p < 0.05$)

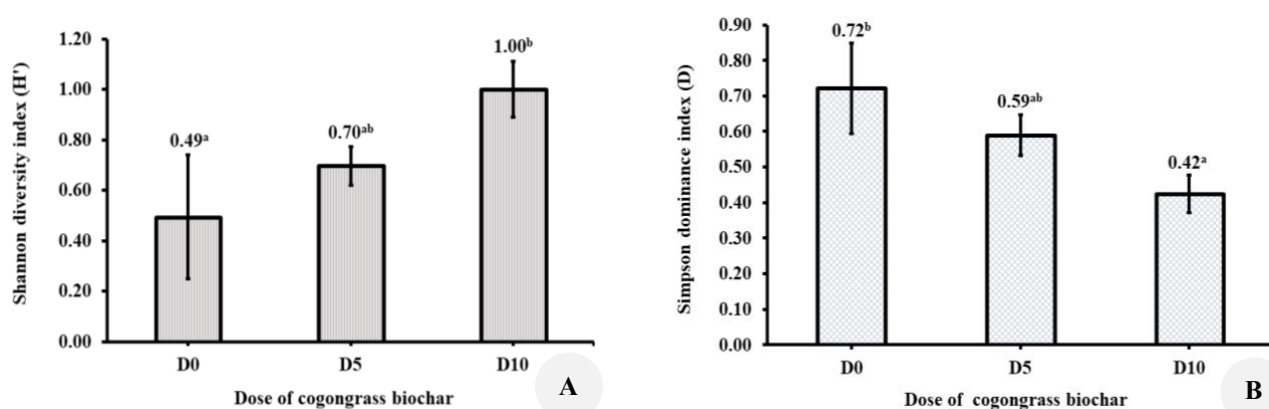


Figure 3. Diversity indices of soil-living invertebrates across cogongrass biochar doses. A. Shannon-Wiener diversity index (H'), and B. Simpson dominance index (D). Bars represent mean ±SD (n = 3). Different letters show significant differences among biochar doses based on Tukey HSD ($p < 0.05$)

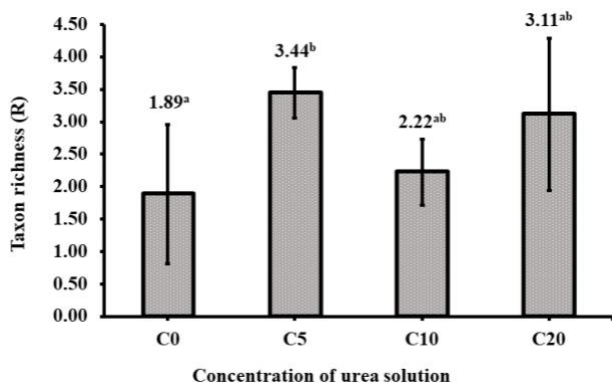


Figure 4. Taxon-level richness of soil-living invertebrates across urea concentrations prepared with *Sargassum* extract. Bars represent mean \pm SD (n = 3). Different letters represent significant differences among urea concentrations based on Tukey HSD ($p < 0.05$)

Discussion

Effect of cogongrass biochar and urea concentration on surface-active invertebrates

Surface-active invertebrates showed taxon-specific responses to cogongrass biochar doses and urea concentrations, while overall taxon-level community diversity indices remained unchanged. Taxon-level richness, Shannon-Wiener diversity, Simpson dominance, and evenness did not differ significantly among treatments. This suggests that the applied amendments did not alter the taxon-level diversity but modified the relative contributions of particular taxa. The pattern implies a reorganization of community structure driven by shifts in the relative contribution of functionally distinct taxa rather than changes in the taxon richness, reflecting differential sensitivities of surface-active taxa to soil amendments (Shimadzu et al. 2015; Menta and Remelli 2020).

The abundance of Diptera, Formicidae, Hymenoptera, and Orthoptera varied in response to biochar and urea inputs, underscoring their sensitivity to changes in soil resource availability and habitat conditions (Menta and Remelli 2020; Vandermeer et al. 2022). Previous studies have shown that cogongrass biochar enriched with urea using *Sargassum* extract can improve soil moisture retention, pH, organic carbon, and nitrogen availability in Ultisol (Kilowasid et al. 2024; 2025). Organic carbon inputs provide an important energy base for soil food webs, and variation in their availability influences population dynamics of soil-dwelling consumers and the predators (Nordberg and Schwarzkopf 2019; Yi et al. 2025). The response of Diptera and non-ant Hymenoptera should be interpreted cautiously, as these taxa are highly mobile and may reflect indirect treatment effects mediated through changes in microhabitat conditions, detrital resources, or prey availability rather than direct soil modification (Santos et al. 2014; Menta and Remelli 2020).

In the present study, Diptera abundance increased at moderate biochar application (5 t ha⁻¹) but declined at higher doses. This suggests that multiple interacting environmental factors regulated the response rather than a

simple linear relationship with biochar input. Although specific drivers were not measured, the non-linear response of Diptera suggests sensitivity to biochar-induced changes in soil habitat conditions, which warrants further investigation (Giagnoni et al. 2019). Accordingly, Diptera responses in this study are interpreted as auxiliary indicators of surface activity rather than primary indicators of soil ecological change. Therefore, conclusions regarding treatment viability are based primarily on less mobile taxa and overall community-level patterns.

Formicidae abundance peaked at the highest biochar dose, reflecting high mobility and broad foraging strategies that allow rapid exploitation of improved habitat conditions and resource availability (Vandermeer et al. 2022; Dejean et al. 2025). Ants are widely recognized as ecosystem engineers in agricultural soil, and the increased abundance may imply enhanced habitat suitability following biochar application (Delgado-Baquerizo et al. 2018). However, the underlying mechanisms driving this response remain unresolved and warrant further investigation using methods that integrate soil structural and trophic analyses (Wang et al. 2021; Liu et al. 2024).

Hymenoptera abundance declined with increasing urea concentrations prepared using *Sargassum* extract. This suggests that excessive nitrogen inputs may negatively affect certain surface-active taxa. The result is consistent with previous reports showing that high nitrogen availability can suppress soil fauna abundance and alter community composition (Hu et al. 2022). A significant interaction between biochar dose and urea concentration was observed only for Orthoptera. This shows that the abundance depended on combined amendment effects rather than single-factor responses. As Orthoptera are mobile herbivores closely related to host plant condition, the response may be associated with combined amendment effects that modify habitat suitability and resource availability, rather than a single-factor response (Leksono et al. 2020; Greyvenstein et al. 2021; Hasibuan et al. 2022).

The delimited number of surface-active taxa showing significant responses suggests that the tested biochar-urea treatments did not broadly disrupt the soil invertebrate community. This selective response pattern shows that enriched cogongrass biochar, when applied at appropriate doses, represents a potentially viable soil management option for soybean agroecosystems in Ultisol-dominated regions of dry land agriculture in Indonesia and other tropical areas with similar soil constraints.

Effects of cogongrass biochar and urea concentration on soil-living invertebrates

Only a subset of soil-living invertebrate taxa responded significantly to the applied treatments, while overall taxon richness remained stable. This pattern suggests that the amendments selectively influenced sensitive functional groups rather than causing widespread community disruption.

Living-soil invertebrate assemblages responded primarily to biochar dose and urea concentration independently. Biochar application significantly increased the abundance of Araneae and Formicidae, while Glossoscolecidae declined with increasing dose. These responses possibly reflect

changes in soil organic carbon content, nutrient availability, and pore structure following biochar incorporation, which may influence microhabitat quality and trophic interactions within the soil matrix (Santos et al. 2014; Michael 2020).

Araneae and Formicidae showed increased abundance at higher biochar doses, suggesting improved habitat conditions and prey availability. Organic carbon inputs support detritivore populations, which sustain higher trophic levels such as generalist predators (Cuff et al. 2022; Mamabolo et al. 2024). Predator-prey interactions among ants, spiders, and other soil invertebrates can contribute to restructuring community composition under biochar-amended conditions (Nyffeler et al. 2017; Nyffeler and Birkhofer 2017; Dejean et al. 2025). In contrast, Glossoscolecidae abundance declined with increasing biochar dose and higher urea concentrations. This may be associated with changes in nutrient balance that constrain earthworm abundance, as reported in stoichiometric studies (Marichal et al. 2011; Zheng et al. 2023). The regulation of soil invertebrate density by C: N:P balance is well established and underscores the importance of stoichiometric constraints in soil food webs (Kilowasid et al. 2014; Tie et al. 2021; Zhang et al. 2022).

Soil-living invertebrate diversity responded positively to biochar application, as signified by increased Shannon-Wiener diversity and reduced Simpson dominance at higher biochar doses. This pattern suggests enhanced community evenness and reduced dominance of single taxa, possibly resulting from increased habitat heterogeneity and micro-niche availability. Improved pore structure and spatial complexity can facilitate coexistence and trophic interactions, supporting more functionally diverse soil communities without reducing taxon richness (Erktan et al. 2020; Maienza et al. 2023).

Limitations and future study directions

Despite the observable contributions, this study has several limitations. The experimental design included limited number of replications, treatment levels, and relatively short observation period, which may not capture long-term or seasonal dynamics of soil invertebrate communities. Furthermore, taxonomic resolution was restricted to selected groups, providing partial representation of overall community composition. Key environmental variables, such as detailed soil microhabitat characteristics and bioactive compound concentrations, were not measured, limiting the mechanistic interpretation of identified patterns. Future studies should incorporate multi-season or multi-year observations and integrate soil physicochemical, biological, and functional trait measurements, alongside finer taxonomic resolution beyond the family level, to better elucidate the ecological mechanisms underlying soil fauna responses to enriched biochar amendments in tropical agroecosystems.

In conclusion, urea-enriched cogongrass biochar prepared with *Sargassum* extract altered the abundance and composition of both surface-active and soil-living invertebrates in an Ultisol soybean agroecosystem, while taxon richness remained largely unchanged. Biochar application was the primary driver of predator responses,

with Formicidae and Araneae increasing at the highest dose. Diptera and Orthoptera showed treatment-specific patterns rather than uniform responses. In the soil-living assemblage, biochar application was associated with higher Shannon-Wiener diversity and lower Simpson dominance, signifying reduced dominance under higher doses. Urea concentration independently increased Formicidae but reduced Glossoscolecidae at specific levels, underscoring that nitrogen inputs shifted soil fauna composition even in the absence of interaction effects. Due to the prevalence of Ultisol and similar constraints across large parts of Indonesia and other tropical regions, these results suggest that enriched cogongrass biochar has broader relevance beyond the study site, provided the application rates are optimized and excessive nitrogen inputs are avoided. The results provide collective evidence that enriched cogongrass biochar can be used to steer soil-fauna community structure in degraded tropical Ultisol without apparent loss of taxa, thereby supporting soil ecological functioning. From a management perspective, optimizing biochar dose and avoiding excessive urea concentrations help maintain beneficial soil fauna while minimizing negative responses of sensitive groups in the soybean agroecosystem. Future studies should test the underlying mechanisms using multi-season monitoring and targeted measurements of soil habitat conditions and trophic pathways, rather than inferring mechanisms from short-term responses.

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