Seasonal and environmental drivers of necrophagous *Diptera* assemblages in tropical agricultural landscapes

Lilian Cristina Rocha Silva[1], Edimar Agnaldo Moreira[2] & Cleiton Antônio Martins[1]

[1] Faculty of Sciences and Technologies of Campos Gerais (FACICA), Campos Gerais, Minas Gerais, Brazil

[2] Federal University of Alfenas (UNIFAL-MG), Institute of Natural Sciences, Alfenas, Minas Gerais, Brazil

Correspondence: Edimar Agnaldo Moreira, Federal University of Alfenas (UNIFAL-MG), Institute of Natural Sciences, Alfenas, Minas Gerais, Brazil. E-mail: edimar.moreira@unifal-mg.edu.br

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Abstract

Carrion-associated *Diptera* play important ecological roles in nutrient cycling and decomposition processes, yet the combined effects of vegetation structure, seasonality, and resource quality on their assemblage organization in tropical agricultural systems remain poorly understood. This study evaluated the influence of vegetation matrix, seasonal variation, and bait type on the abundance, richness, diversity, and community composition of necrophagous *Diptera* in agricultural landscapes of southeastern Brazil. Sampling followed a completely randomized factorial design combining two vegetation matrices, coffee plantations and pasturelands, with two bait types, bovine liver and fish, across dry and rainy seasons, totaling 64 independent sampling units. Traps remained exposed for 48 h, and collected specimens were identified to family level and classified into morphospecies. Community structure was evaluated using diversity indices, factorial analyses, Tukey post-hoc tests, and multivariate analyses based on Bray–Curtis dissimilarity, including NMDS and PERMANOVA. A total of 472 individuals distributed among 13 morphospecies and seven *Diptera* families were recorded. Coffee plantations exhibited higher abundance and diversity values than pasture areas, particularly during the rainy season, whereas bovine liver showed greater attractiveness than fish bait. The highest diversity values were observed in coffee plantations baited with bovine liver during the rainy season, suggesting synergistic interactions among vegetation complexity, climatic conditions, and resource quality. Multivariate analyses revealed significant differences in community composition among treatments. Overall, vegetation structure, seasonality, and bait type jointly influenced the organization of necrophagous *Diptera* assemblages under the evaluated tropical agricultural conditions.

Keywords: carrion ecology, forensic entomology, habitat heterogeneity, necrophagous *Diptera*, tropical agricultural landscapes.

Determinantes sazonais e ambientais das comunidades de *Diptera* necrófagos em paisagens agrícolas tropicais

Resumo

Os dípteros necrófagos desempenham importantes funções ecológicas relacionadas à decomposição da matéria orgânica e ciclagem de nutrientes, porém os efeitos combinados da estrutura da vegetação, sazonalidade e qualidade do recurso sobre a organização dessas assembleias em sistemas agrícolas tropicais ainda são pouco compreendidos. O presente estudo avaliou a influência da matriz vegetal, variação sazonal e tipo de isca sobre a abundância, riqueza, diversidade e composição de comunidades de *Diptera* necrófagos em paisagens agrícolas do sul de Minas Gerais, Brasil. A amostragem seguiu um delineamento inteiramente casualizado em esquema fatorial combinando duas matrizes vegetais, cafezais e pastagens, dois tipos de isca, fígado bovino e peixe, e duas estações sazonais, seca e chuvosa, totalizando 64 unidades amostrais independentes. As armadilhas permaneceram expostas por 48 h, sendo os espécimes coletados identificados em nível de família e classificados em morfoespécies. A estrutura das comunidades foi avaliada por índices de diversidade, análises fatoriais, testes de *Tukey* e análises multivariadas baseadas em dissimilaridade de *Bray–Curtis*, incluindo *NMDS* e *PERMANOVA*. Foram registrados 472 indivíduos distribuídos em 13 morfoespécies e sete famílias de *Diptera*. Cafezais apresentaram maiores valores de abundância e diversidade em comparação às áreas de pastagem, especialmente durante o período chuvoso, enquanto o fígado bovino apresentou maior atratividade em relação à isca de peixe. Os maiores valores de diversidade foram observados em cafezais iscados com fígado bovino durante a estação chuvosa, sugerindo interações sinérgicas entre complexidade vegetal, condições climáticas e qualidade do recurso. As análises multivariadas revelaram diferenças significativas na composição das assembleias entre os tratamentos avaliados. De maneira geral, a estrutura da vegetação, sazonalidade e tipo de isca influenciaram conjuntamente a organização das assembleias de *Diptera* necrófagos em ambientes agrícolas tropicais.

Palavras-chave: ecologia de carcaças, entomologia forense, heterogeneidade ambiental, *Diptera* necrófagos, paisagens agrícolas tropicais.

1. Introduction

Carrion decomposition represents a fundamental ecological process responsible for nutrient recycling, energy transfer, and maintenance of trophic interactions in terrestrial ecosystems. This process is strongly mediated by necrophagous insects, especially *Diptera*, which rapidly colonize decomposing organic matter and influence decomposition dynamics across environmental gradients (Barton et al., 2013; Benbow et al., 2019). In addition to their ecological importance, carrion-associated *Diptera* are widely recognized as key organisms in forensic entomology due to their applicability in postmortem interval estimation and reconstruction of decomposition processes under distinct environmental conditions (Amendt et al., 2011; Tomberlin et al., 2011).

The organization of necrophagous *Diptera* assemblages is influenced by multiple interacting environmental factors operating at both local and landscape scales. Vegetation structure, habitat complexity, microclimatic stability, and resource availability are among the main drivers affecting insect colonization, persistence, dispersal, and community composition in agricultural and fragmented ecosystems (Fahrig, 2017; Tscharntke et al., 2012). Structurally simplified environments, such as pasturelands, are commonly associated with increased solar radiation, reduced humidity retention, and greater temperature fluctuations, conditions that may negatively affect carrion-associated insect diversity and activity. Conversely, more heterogeneous agricultural matrices, including perennial crops with greater vegetation cover, may provide favorable microclimatic conditions and facilitate the maintenance of more diverse insect assemblages (Prevedello; Vieira, 2010).

Seasonality also plays a central role in shaping necrophagous insect communities in tropical ecosystems. Variations in temperature, rainfall, humidity, and decomposition dynamics across dry and rainy seasons directly influence *Diptera* abundance, successional patterns, and resource exploitation strategies (Sharanowski et al., 2008; Cruz et al., 2021). Recent studies conducted in tropical and subtropical environments have demonstrated that carrion-associated *Diptera* communities may exhibit strong temporal turnover, with marked differences in species composition and successional trajectories among seasons and habitat types (Oliveira-Costa; Vasconcelos, 2021; Wang et al., 2022).

Resource quality represents another major ecological filter affecting necrophagous *Diptera* assemblages. Protein-rich substrates release volatile organic compounds during decomposition that mediate insect attraction and colonization behavior (Dekeirsschieter et al., 2009; Paczkowski; Schütz, 2011). Differences in bait composition may therefore alter abundance patterns, diversity, and assemblage organization by influencing both detectability and resource suitability for necrophagous insects. Experimental studies have demonstrated that distinct carrion substrates can significantly modify community structure and ecological succession patterns of *Diptera* in decomposing systems (Benbow et al., 2015; Wang et al., 2022).

Despite advances in carrion ecology and forensic entomology, ecological studies simultaneously integrating seasonal variation, vegetation structure, and resource type in tropical agricultural landscapes remain relatively scarce, particularly in Neotropical regions. Most available studies are restricted to short-term surveys or single-environment approaches, limiting broader ecological interpretations regarding the mechanisms structuring necrophagous *Diptera* assemblages across heterogeneous agricultural matrices (Barton et al., 2013; Benbow et al., 2019). Furthermore, baseline ecological data remain insufficient for many tropical regions, including southeastern Brazil, where agricultural expansion and habitat modification may directly influence carrion-associated insect dynamics (Fahrig, 2017).

In this context, the present study evaluated the effects of seasonal variation, vegetation complexity, and resource type on the abundance, richness, diversity, and community composition of necrophagous *Diptera* assemblages in tropical agricultural landscapes of southeastern Brazil. We hypothesized that: (i) structurally complex environments support more diverse and stable *Diptera* assemblages due to increased environmental heterogeneity and microclimatic buffering; (ii) seasonal variation significantly alters community composition and abundance patterns; and (iii) protein-rich resources exhibit greater attractiveness and influence assemblage structuring under contrasting environmental conditions.

2. Materials and Methods

2.1 Study area

The study was conducted in agricultural landscapes located in the municipalities of Campo do Meio and Campos Gerais, southern Minas Gerais State, southeastern Brazil. Both municipalities are situated within an important coffee-producing region characterized by agricultural mosaics composed mainly of coffee plantations, pasturelands, and remnant fragments of Atlantic Forest vegetation.

Campo do Meio (21°25′45″ S and 45°49′39″ W) presents altitudes ranging from 780 to 1,083 m and a tropical savanna climate (*Aw*), with dry winters and rainy summers. Campos Gerais (21°14′06″ S and 45°45′32″ W) is located at approximately 843 m above sea level and is characterized by extensive coffee plantations associated with pasture areas and native vegetation remnants.

Sampling areas in both municipalities included pasturelands with low vegetation complexity and high environmental exposure, as well as structurally more complex coffee plantations presenting greater canopy cover, humidity retention, and microclimatic stability. The proximity between agricultural matrices and forest remnants created heterogeneous environmental conditions potentially influencing the abundance, diversity, and community organization of necrophagous *Diptera* assemblages. Sampling campaigns were conducted during dry and rainy seasons under comparable environmental conditions in order to evaluate seasonal and environmental effects on carrion-associated *Diptera* communities in tropical agricultural landscapes.

2.2 Experimental design

The study followed a completely randomized 2 × 2 × 2 factorial design composed of three explanatory factors: vegetation matrix (coffee plantation and pasture), bait type (bovine liver and fish), and seasonality (rainy and dry seasons). The combination of these factors generated eight treatment groups (CLR, CFR, PLR, PFR, CLD, CFD, PLD, and PFD), totaling 64 independent sampling units. The vegetation matrix included two agricultural environments with contrasting structural complexity, namely coffee plantations and pasturelands, while seasonality included dry and rainy seasons. Sampling was conducted in agricultural areas located in Campo do Meio and Campos Gerais, Minas Gerais State, Southeastern Brazil.

Within each municipality, sampling units were distributed in both pasture and coffee plantation matrices. Traps were installed at independent sampling points separated by a minimum distance of 10 m to reduce spatial interference among attractants and ensure sampling independence. All traps remained simultaneously active during the same exposure interval under comparable environmental conditions. Sampling effort was expanded in relation to previous surveys through the inclusion of additional sampling areas, seasonal replicates, and increased trap number, allowing broader evaluation of spatial and temporal patterns affecting necrophagous *Diptera* assemblages in tropical agricultural landscapes.

2.3 Trap construction and bait preparation

Sampling traps were constructed using 2-L polyethylene terephthalate (PET) bottles measuring approximately 20 cm in height and 9 cm in diameter. Two lateral openings were made in the lower third of each trap to allow insect entry. An inverted funnel system constructed from the bottle neck was installed in the upper portion of the trap to facilitate insect retention and reduce escape probability after capture. To minimize potential visual attraction bias, all traps were externally coated in black, simulating low-light conditions commonly associated with decomposing organic substrates. Traps were suspended using nylon cords at approximately 1.8 m above ground level and remained protected from direct contact with the soil during the exposure period.

Two bait types were used as necrophagous attractants: bovine liver and fish tissue. Approximately 100 g of bait were placed inside each trap at the time of installation. Bovine liver was selected as a protein-rich substrate with rapid decomposition potential, whereas fish tissue represented an alternative organic resource with distinct volatile and decomposition characteristics. All bait material was used fresh and remained exposed in the traps throughout the 48-h sampling period without replacement.

2.4 Sampling procedure

Sampling campaigns were conducted between October 2025 and May 2026 during both dry and rainy seasons under comparable environmental conditions. In each campaign, traps were simultaneously installed in pasturelands and coffee plantations located in the municipalities of Campo do Meio and Campos Gerais, Minas Gerais State, Southeastern Brazil. Sampling points were distributed along environmental gradients associated with vegetation structure and habitat complexity. Traps were positioned at independent sampling sites separated by a minimum distance of 10 m in order to minimize interference among attractants and ensure sampling independence. All traps remained suspended at approximately 1.8 m above ground level and exposed in the field for 48 h without bait replacement.

The expanded sampling effort consisted of 64 independent sampling units distributed among municipalities, vegetation matrices, bait types, and seasonal replicates. Multiple sampling campaigns were performed throughout the study period in order to reduce temporal bias and increase ecological representativeness across contrasting environmental conditions. After each exposure interval, captured insects were carefully removed from the traps, individually stored, and transported to the laboratory for sorting, identification, and subsequent ecological analyses.

2.5 Specimen processing and identification

Collected specimens were transported to the laboratory and sorted under a stereomicroscope for subsequent identification and classification. Individuals were initially identified to family level using standard taxonomic keys for *Diptera* (Carrera, 1967; Carvalho & Queiroz, 2003). Whenever possible, specimens were further identified to morphospecies level based on external morphological characteristics, including body coloration, wing venation patterns, bristle arrangement, and body size.

Due to the taxonomic complexity and high diversity commonly associated with necrophagous *Diptera* assemblages in tropical environments, the use of morphospecies was adopted as an operational approach for ecological analyses. This methodology is widely employed in ecological studies involving hyperdiverse insect groups when complete taxonomic resolution is limited. After identification, specimens were quantified and organized according to municipality, vegetation matrix, season, and bait type for subsequent ecological and statistical analyses. Representative specimens were preserved and deposited in a reference collection for future taxonomic confirmation and comparative studies.

2.6 Diversity and community analysis

Community structure was evaluated using ecological diversity metrics, including species richness (S), Shannon diversity index (H′), and Pielou's evenness index (J′). Species richness corresponded to the total number of morphospecies recorded in each treatment, whereas Shannon diversity and evenness were used to evaluate diversity patterns and the distribution uniformity of individuals among morphospecies.

Sampling sufficiency was assessed through species accumulation curves based on cumulative morphospecies richness across sampling units. The stabilization tendency of the accumulation curves was used as an indicator of sampling adequacy under the evaluated environmental conditions. Community composition patterns among municipalities, vegetation matrices, seasons, and bait types were further investigated using Bray–Curtis dissimilarity matrices. Multivariate analyses were employed to evaluate ecological similarities and compositional differences among necrophagous *Diptera* assemblages across spatial and temporal environmental gradients.

2.7 Statistical analysis

Differences in abundance, richness, and Shannon diversity of necrophagous *Diptera* assemblages were evaluated within a completely randomized $2 \times 2 \times 2$ factorial framework, considering vegetation matrix (coffee plantation and pasture), bait type (bovine liver and fish), and seasonality (rainy and dry seasons) as fixed factors. Municipalities were treated as sampling locations used to increase spatial replication and were not included as factors in the statistical models because preliminary analyses indicated no significant municipality effect.

Before inferential analyses, data were evaluated for compliance with model assumptions. Normality of residuals was assessed using the Shapiro–Wilk test, whereas homogeneity of variances was evaluated using Levene's test. When assumptions were met, three-way analyses of variance (ANOVA) were performed to test the main effects of vegetation matrix, bait type, seasonality, and their interactions on abundance and Shannon diversity. Significant effects were further explored using *Tukey's* HSD post-hoc tests for pairwise comparisons among treatment means.

Sampling sufficiency was assessed through species accumulation curves based on cumulative morphospecies richness across sampling units. Observed richness was compared with non-parametric richness estimators to evaluate the completeness of sampling effort under the studied environmental conditions. Community composition patterns were investigated using Bray–Curtis dissimilarity matrices calculated from morphospecies abundance data. Non-metric multidimensional scaling (NMDS) was used to visualize differences in assemblage composition among treatments. Statistical differences in community composition were tested using permutational multivariate analysis of variance (PERMANOVA) with 999 permutations. Before PERMANOVA, homogeneity of multivariate dispersion among groups was evaluated using the betadisper procedure to verify whether differences among treatments reflected compositional variation rather than differences in within-group dispersion. All statistical analyses were performed in R version 4.3.1 (R Core Team, 2023) using the packages *vegan*, *stats*, *car*, *agricolae*, and *ggplot2*. Statistical significance was considered at $p < 0.05$.

3. Results

3.1 Species richness and sampling sufficiency

During the sampling period, a total of 472 individuals of necrophagous *Diptera* were collected, representing 13 morphospecies distributed among seven families. The distribution of morphospecies among municipalities, vegetation matrices, seasons, and bait types is presented in Supplementary (Table S1). Overall abundance differed among treatments, with higher numbers of individuals recorded in coffee plantations (N = 299; 63.3%) compared to pasture areas (N = 173; 36.7%). In relation to bait type, bovine liver accounted for most captured individuals (N = 286; 60.6%), whereas fish bait accounted for 186 individuals (39.4%) (Figure 1C; Table 1). Species richness also varied among treatments. Coffee plantations baited with bovine liver during the rainy season exhibited the highest richness values (S = 13 morphospecies), whereas pasture areas baited with fish during the dry season showed the lowest richness (S = 5 morphospecies) (Table 1). Intermediate richness values were observed in pasture areas baited with bovine liver (S = 8) and coffee plantations baited with fish (S = 9).

Table 1. Community metrics of necrophagous *Diptera* assemblages among treatments in southeastern Brazilian agricultural landscapes.

Treatment	Mean abundance per sampling unit (± SD)	N (individuals)	Richness (S)	Shannon (H′)	Evenness (J′)
Coffee + Liver + Rainy season (CLR)	42.0 ± 4.2	98	13	2.34 ± 0.06	0.93
Coffee + Fish + Rainy season (CFR)	28.4 ± 3.1	79	9	1.98 ± 0.05	0.86
Pasture + Liver + Rainy season (PLR)	24.7 ± 2.8	71	8	1.72 ± 0.04	0.81
Pasture + Fish + Rainy season (PFR)	12.5 ± 1.9	35	6	1.14 ± 0.05	0.72
Coffee + Liver + Dry season (CLD)	30.1 ± 3.4	70	10	1.91 ± 0.05	0.88
Coffee + Fish + Dry season (CFD)	18.9 ± 2.3	52	7	1.54 ± 0.04	0.79
Pasture + Liver + Dry season (PLD)	16.3 ± 2.1	47	6	1.31 ± 0.04	0.73
Pasture + Fish + Dry season (PFD)	7.0 ± 1.7	20	5	0.88 ± 0.03	0.64

Note: N = total number of individuals collected per treatment; S = morphospecies richness; H′ = Shannon diversity index; J' = Pielou's evenness index. CLR = coffee plantation baited with bovine liver during the rainy

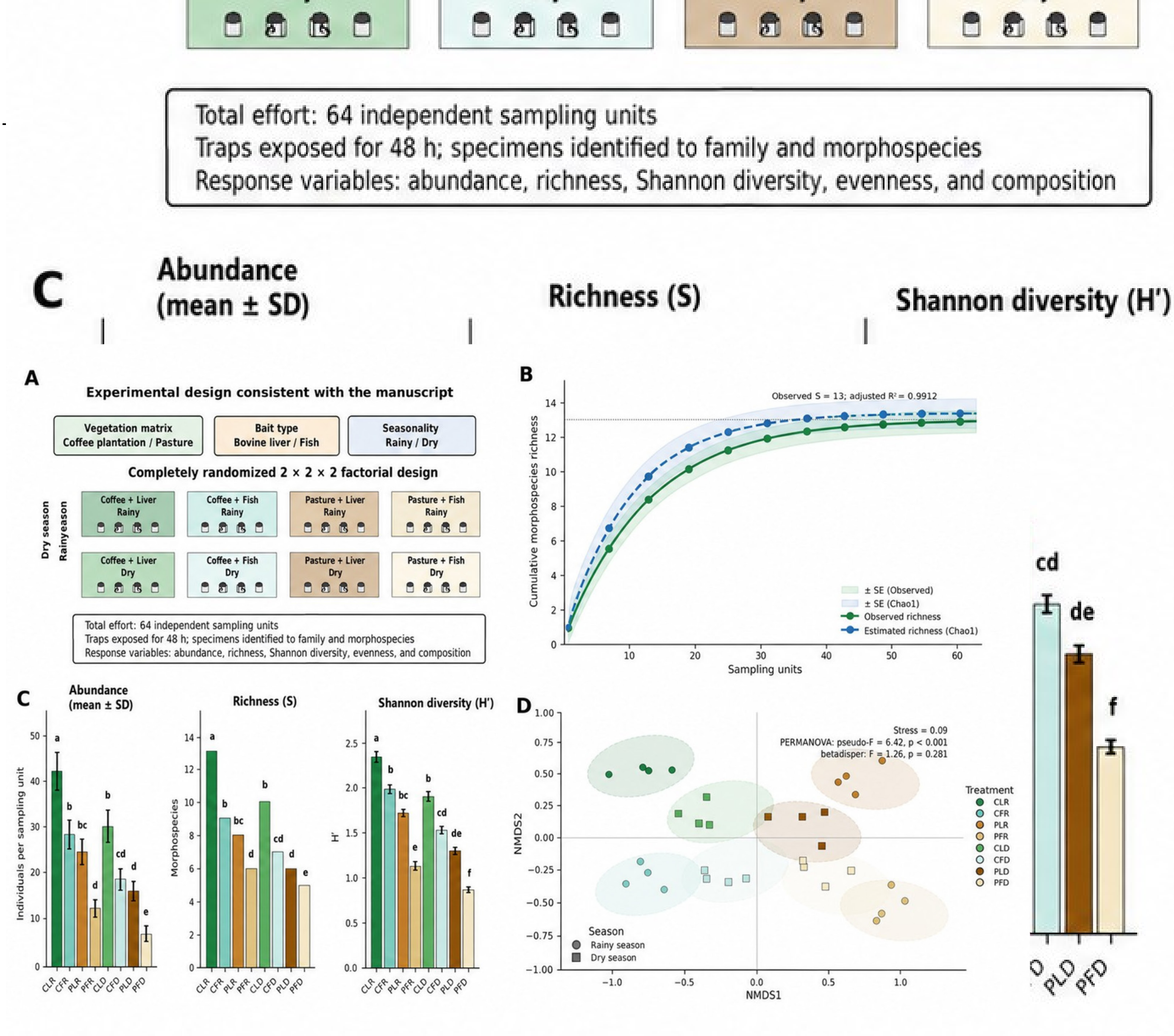

Figure 1. Experimental design, sampling sufficiency, diversity metrics, and community composition of necrophagous *Diptera* assemblages in tropical agricultural landscapes of southeastern Brazil. (**A**) Schematic representation of the completely randomized 2 × 2 × 2 factorial design combining two vegetation matrices (coffee plantation and pasture), two bait types (bovine liver and fish), and two seasonal periods (rainy and dry seasons), totaling 64 independent sampling units. Traps remained exposed for 48 h, and collected specimens were identified to family and morphospecies levels. (**B**) Species accumulation curves showing observed and Chao1-estimated cumulative morphospecies richness across sampling units. Shaded areas represent ± standard error. The horizontal dotted line indicates observed total richness (S = 13), and the adjusted coefficient of determination (adjusted R^2 = 0.9912) indicates adequate sampling coverage. (**C**) Mean abundance (± SD), morphospecies richness (S), and Shannon diversity index (H') among treatments combining vegetation matrix, bait type, and seasonality. Different letters above bars indicate significant differences among treatments according to Tukey's HSD test ($p < 0.05$). Treatments: CLR = coffee plantation + bovine liver + rainy season; CFR = coffee plantation + fish + rainy season; PLR = pasture + bovine liver + rainy season; PFR = pasture + fish + rainy season; CLD = coffee plantation + bovine liver + dry season; CFD = coffee plantation + fish + dry season; PLD = pasture + bovine liver + dry season; PFD = pasture + fish + dry season. (**D**) Non-metric multidimensional scaling (NMDS) ordination based on Bray–Curtis dissimilarity showing differences in community composition among treatments. Symbols represent seasons (circles = rainy season; squares = dry season), and shaded ellipses represent treatment groupings. PERMANOVA and betadisper results are shown within the panel. Source: Authors, 2026.

The species accumulation curve exhibited a rapid increase in cumulative morphospecies richness during the initial sampling campaigns, followed by a stabilization tendency after approximately the 42nd sampling unit, indicating satisfactory sampling sufficiency under the evaluated environmental conditions (Figure 1B). The relationship between sampling effort and cumulative richness showed a high adjusted coefficient of determination (adjusted $R^2 = 0.9912$), suggesting that most morphospecies associated with the evaluated environmental gradients were successfully sampled.

Residual diagnostics indicated no significant deviation from model assumptions. The *Shapiro–Wilk* test indicated normal distribution of residuals ($W = 0.97$, $p = 0.6214$), and no evidence of residual autocorrelation was detected according to the *Box–Pierce* test ($X^2 = 1.37$, $p = 0.2418$). In addition, factorial analyses revealed significant effects of vegetation matrix, seasonality, and bait type on abundance patterns (Table 2), reinforcing the observed spatial and temporal differences among treatments.

Although sampling sufficiency was supported by the stabilization tendency of the accumulation curves, the environmental heterogeneity and seasonal variation observed among agricultural matrices should be considered when interpreting broader ecological patterns affecting necrophagous *Diptera* assemblages in tropical landscapes.

3.2 Effects of vegetation matrix, seasonality, and bait type on diversity patterns

Vegetation matrix and seasonality influenced the diversity patterns of necrophagous *Diptera* assemblages. Coffee plantations presented higher Shannon diversity values ($H' = 2.11 \pm 0.09$) compared to pasture areas ($H' = 1.53 \pm 0.07$) (Figure 1C; Table 1). Pielou's evenness index also showed higher values in coffee plantations ($J' = 0.87$) than in pasture areas ($J' = 0.74$), indicating a more balanced distribution of individuals among morphospecies in structurally more complex environments.

Seasonal variation also affected community diversity patterns. Sampling campaigns conducted during the rainy season exhibited higher Shannon diversity values ($H' = 2.24 \pm 0.08$) and greater abundance compared to dry-season campaigns ($H' = 1.46 \pm 0.06$), suggesting that environmental conditions associated with higher humidity and temperature stability favored necrophagous *Diptera* activity and assemblage organization.

Three-way ANOVA revealed significant main effects of vegetation matrix, seasonality, and bait type on abundance and diversity metrics (Table 2). Significant interaction effects were also detected, indicating that the response of necrophagous *Diptera* assemblages depended on the combined influence of environmental structure, climatic conditions, and resource type. Vegetation matrix significantly affected Shannon diversity ($F = 11.84$, $p = 0.004$), whereas seasonality exhibited strong effects on both abundance ($F = 18.92$, $p < 0.001$) and diversity ($F = 15.37$, $p = 0.001$). Bait type also significantly influenced abundance patterns ($F = 21.46$, $p < 0.001$), with bovine liver consistently attracting more individuals than fish bait. Significant interaction effects were detected between vegetation matrix and seasonality ($F = 5.21$, $p = 0.031$), vegetation matrix and bait type ($F = 4.76$, $p = 0.039$), and seasonality and bait type ($F = 4.18$, $p = 0.048$) for abundance patterns (Table 2). These results indicate that the ecological responses of necrophagous *Diptera* were influenced not only by individual environmental factors but also by their combined effects.

Table 2. Effects of vegetation matrix, seasonality, bait type, and their interactions on abundance and Shannon diversity (H') of necrophagous *Diptera* assemblages: results of a three-way ANOVA.

Response variable	Factor	df	F	*p*
Abundance	Vegetation matrix	1	11.84	0.004
Abundance	Seasonality	1	18.92	< 0.001
Abundance	Bait type	1	21.46	< 0.001
Abundance	Matrix × seasonality	1	5.21	0.031
Abundance	Matrix × bait	1	4.76	0.039
Abundance	Seasonality × bait	1	4.18	0.048
Abundance	Matrix × seasonality × bait	1	3.92	0.056
Shannon diversity (H′)	Vegetation matrix	1	10.37	0.006
Shannon diversity (H′)	Seasonality	1	15.37	0.001

Response variable	Factor	df	F	*p*
Shannon diversity (H′)	Bait type	1	13.84	0.002
Shannon diversity (H′)	Matrix × seasonality	1	4.69	0.041
Shannon diversity (H′)	Matrix × bait	1	4.12	0.050
Shannon diversity (H′)	Seasonality × bait	1	3.87	0.058
Shannon diversity (H′)	Matrix × seasonality × bait	1	3.24	0.084

Note: Factorial ANOVA evaluating the effects of vegetation matrix, seasonality, bait type, and their interactions on abundance and Shannon diversity of necrophagous *Diptera* assemblages. Significant values ($p < 0.05$) indicate effects of environmental structure, seasonal variation, resource type, or their interactions on assemblage organization under tropical agricultural conditions. Source: Authors, 2026.

Post-hoc comparisons using *Tukey's* HSD test indicated that coffee plantations baited with bovine liver during the rainy season exhibited significantly higher abundance and diversity values than pasture areas baited with fish during the dry season ($p < 0.05$). Intermediate treatments, including pasture areas baited with bovine liver and coffee plantations baited with fish, showed intermediate diversity values and did not differ consistently among all treatment combinations.

Bait type also influenced abundance and diversity patterns. Traps baited with bovine liver exhibited higher Shannon diversity values (H′ = 2.18 ± 0.08) than traps baited with fish (H′ = 1.32 ± 0.06) (Figure 1C; Table 1). Similarly, evenness values were higher in liver-baited treatments (J′ = 0.89) compared to fish-baited treatments (J′ = 0.71). In terms of abundance, bovine liver attracted approximately 2.1 times more individuals than fish bait (Figure 1C; Table 1). Overall, the observed patterns support the results of the three-way ANOVA, demonstrating that vegetation structure, seasonality, and bait type jointly influenced the diversity, abundance, and distribution of necrophagous *Diptera* assemblages under the evaluated tropical agricultural conditions.

3.3 Combined effects of vegetation matrix, seasonality, and bait type

When vegetation matrix, seasonality, and bait type were analyzed jointly, differences in abundance, richness, diversity, and community composition patterns were observed among treatments. Coffee plantations baited with bovine liver during the rainy season presented the highest abundance and diversity values (N = 98 individuals; S = 13; H′ = 2.34; J′ = 0.93), whereas pasture areas baited with fish during the dry season showed the lowest values (N = 20 individuals; S = 5; H′ = 0.88; J′ = 0.64). Intermediate values were recorded in pasture areas baited with bovine liver during the rainy season (N = 71; S = 8; H′ = 1.72; J′ = 0.81) and coffee plantations baited with fish during the rainy season (N = 79; S = 9; H′ = 1.98; J′ = 0.86) (Figure 1C; Table 1).

The interaction among vegetation matrix, seasonality, and bait type was supported by factorial analyses, indicating that diversity patterns varied according to the combination of environmental structure, climatic conditions, and bait resource (Table 2). Treatments associated with coffee plantations and bovine liver during the rainy season consistently exhibited higher abundance and diversity values than treatments associated with pasture areas and fish bait during the dry season.

Community composition analyses revealed clear separation tendencies among treatments in reduced multivariate space (Figure 1D). The NMDS ordination provided an adequate representation of assemblage composition patterns (stress = 0.09), particularly distinguishing dry- and rainy-season assemblages as well as contrasting vegetation matrices and bait types. PERMANOVA analysis detected significant differences in community composition among treatments (pseudo-F = 6.42, $p < 0.001$), indicating that vegetation structure, seasonality, and bait type jointly influenced assemblage organization.

The homogeneity of multivariate dispersion analysis indicated no significant differences in within-group dispersion patterns (betadisper: F = 1.26, $p = 0.281$), supporting the interpretation that the PERMANOVA results primarily reflected compositional differences among treatments rather than heterogeneity in dispersion patterns. Overall, the observed results suggest that vegetation structure, seasonal variation, and resource quality jointly influenced the organization, diversity, and composition of necrophagous *Diptera* assemblages under the evaluated tropical agricultural conditions.

4. Discussion

4.1 Species richness and sampling sufficiency

The richness recorded in the present study, represented by 13 morphospecies distributed among seven *Diptera* families, together with the stabilization tendency observed in the species accumulation curve (Figure 1B), suggests that the expanded sampling effort was sufficient to characterize the main ecological patterns of necrophagous *Diptera* assemblages under the evaluated agricultural conditions. Rapid increases followed by asymptotic stabilization are commonly observed in carrion-associated insect communities due to the ephemeral nature of decomposing resources and the rapid colonization dynamics characteristic of necrophagous Diptera (Barton et al., 2013; Benbow et al., 2015).

Compared to preliminary observations previously conducted in Campo do Meio (Moreira et al., 2014), the present study substantially expanded spatial and temporal sampling effort through the inclusion of additional municipalities, seasonal replicates, and increased sampling units, allowing broader ecological interpretation of carrion-associated *Diptera* responses across tropical agricultural landscapes.

Higher abundance values recorded in coffee plantations compared to pasture areas suggest that structurally more complex agricultural matrices may provide more favorable environmental conditions for necrophagous *Diptera* assemblages. Vegetation complexity is frequently associated with increased habitat heterogeneity, microclimatic stability, and reduced environmental exposure, factors capable of influencing insect movement, persistence, and resource detection within agricultural landscapes (Fahrig, 2017; Tscharntke et al., 2012).

In contrast, simplified pasture environments are generally characterized by greater solar exposure, lower humidity retention, stronger wind incidence, and higher temperature fluctuations, conditions that may negatively affect *Diptera* activity and community organization (Prevedello; Vieira, 2010). The lower abundance and richness observed in pasture matrices reinforce the hypothesis that environmental simplification may reduce habitat suitability for carrion-associated insects.

Although the species accumulation curve indicated satisfactory sampling sufficiency, the use of morphospecies as operational taxonomic units and the restricted temporal scale of the study should be considered when interpreting broader ecological patterns. Nevertheless, the observed results provide consistent evidence of local assemblage responses to environmental heterogeneity within tropical agricultural matrices.

4.2 Effects of vegetation matrix and seasonality on diversity

The higher diversity and evenness values observed in coffee plantations suggest that structurally more complex agricultural environments may favor the organization of more balanced necrophagous *Diptera* assemblages. Environments with greater vegetation heterogeneity generally provide increased habitat complexity, humidity retention, and microclimatic buffering, factors that may contribute to the maintenance of insect diversity within human-modified landscapes (Fahrig, 2017; Tscharntke et al., 2012).

In the present study, coffee plantations exhibited higher Shannon diversity and more uniform morphospecies distribution than pasture areas (Figure 1C; Table 1), indicating lower dominance patterns and greater assemblage balance under structurally heterogeneous conditions. Similar ecological responses have been reported for insect communities occupying fragmented agricultural landscapes, where vegetation structure directly influences richness, diversity, and functional organization through its effects on environmental stability and resource accessibility (Moretti et al., 2017; Seibold et al., 2019).

Seasonality also strongly influenced diversity patterns. Rainy-season campaigns exhibited substantially higher abundance and diversity values than dry-season campaigns, suggesting that increased humidity and thermal stability may favor flight activity, resource detection, and colonization efficiency of necrophagous *Diptera*. Climatic conditions associated with precipitation are known to influence decomposition dynamics, volatile compound dispersion, and insect succession patterns in carrion-associated communities (Benbow et al., 2015; Wang et al., 2022).

In contrast, dry-season conditions may intensify environmental stress through increased temperature fluctuations and reduced humidity, potentially limiting the activity and persistence of some *Diptera* groups. The separation tendency observed between dry- and rainy-season assemblages in the NMDS ordination further supports the importance of temporal environmental variation in shaping community organization patterns.

Although the observed results indicate strong associations between vegetation structure, seasonality, and necrophagous *Diptera* diversity, the use of morphospecies and the absence of species-level taxonomic resolution should be considered when interpreting fine-scale ecological responses. Even so, the data strongly suggest that environmental heterogeneity represents an important driver affecting carrion-associated *Diptera* assemblages in tropical agricultural environments.

4.3 Effects of bait type and resource quality

Bait type strongly influenced abundance, richness, and diversity patterns of necrophagous *Diptera* under the evaluated agricultural conditions. Traps baited with bovine liver consistently exhibited higher abundance, richness, Shannon diversity, and evenness values than fish-baited traps (Figure 1C; Table 1), indicating differences in attractiveness among decomposing substrates.

Protein-rich tissues are known to release large quantities and greater diversity of volatile organic compounds during decomposition, increasing resource detectability and attractiveness for necrophagous insects (Dekeirsschieter et al., 2009; Paczkowski & Schütz, 2011). These volatile compounds function as essential olfactory cues guiding *Diptera* flight orientation and colonization behavior, particularly in environments where ephemeral carrion resources must be rapidly detected.

The higher abundance observed in liver-baited traps suggests that bovine liver represented a more energetically favorable or chemically attractive resource under the evaluated tropical conditions. Similar responses have been reported in carrion ecology studies demonstrating that substrate composition can strongly influence insect attraction intensity and community organization (Benbow et al., 2015; Wang et al., 2022).

From a behavioral perspective, these patterns are also consistent with ecological theories related to optimal resource exploitation, in which insects tend to preferentially colonize substrates maximizing energetic gain and reproductive opportunities (Pyke, 1984). Because necrophagous *Diptera* depend heavily on volatile chemical signals for locating ephemeral resources, differences in decomposition chemistry among bait types likely contributed directly to the abundance and diversity patterns observed among treatments.

Nevertheless, because only two bait types were evaluated, broader conclusions regarding substrate preference should be interpreted cautiously. Future studies incorporating additional carrion substrates and decomposition stages may further improve understanding of resource selection dynamics in tropical necrophagous *Diptera* assemblages.

4.4 Combined effects and community structuring

The combined analysis of vegetation matrix, seasonality, and bait type revealed clear differences in abundance, diversity, and community composition among treatments, consistent with the significant interaction effects detected in factorial analyses (Table 2). Coffee plantations baited with bovine liver during the rainy season consistently presented the highest abundance and diversity values, whereas pasture areas baited with fish during the dry season exhibited the lowest community metrics.

These patterns indicate that structurally heterogeneous environments associated with highly attractive protein-rich resources may favor *Diptera* colonization and coexistence patterns within agricultural landscapes. Vegetation complexity can influence environmental stability, odor plume dispersion, and habitat suitability, potentially facilitating resource detection and persistence of necrophagous insects (Fahrig, 2017; Tscharntke et al., 2012). Simultaneously, differences in bait composition may alter volatile compound release and resource attractiveness, influencing colonization intensity and assemblage composition (Dekeirsschieter et al., 2009; Paczkowski & Schütz, 2011).

The NMDS ordination demonstrated clear separation tendencies among treatments (Figure 1D), particularly between dry- and rainy-season assemblages and among contrasting vegetation matrices and bait types. PERMANOVA analyses further supported the existence of significant compositional differences among treatments, indicating that environmental filtering and resource quality jointly influenced community structuring. In addition, the absence of significant differences in multivariate dispersion suggests that the observed PERMANOVA patterns primarily reflected ecological structuring rather than stochastic within-group variability.

The interaction among vegetation structure, climatic conditions, and resource quality observed in the present study is consistent with ecological frameworks proposing that community assembly results from the combined effects of habitat filtering and resource availability (Benbow et al., 2015; Barton et al., 2013). Under structurally complex conditions, the availability of highly attractive carrion resources may increase colonization opportunities and promote coexistence among necrophagous *Diptera* morphospecies.

Although the present study was based on morphospecies classification and restricted temporal sampling, the observed results provide strong evidence that vegetation structure, seasonality, and bait composition jointly influence carrion-associated *Diptera* assemblages within tropical agricultural systems.

4.5 Ecological implications

The present study demonstrates that necrophagous *Diptera* assemblages in tropical agricultural landscapes are strongly influenced by environmental heterogeneity and resource quality. Differences observed among vegetation matrices, seasons, and bait types indicate that both habitat structure and carrion characteristics can substantially affect abundance, diversity, and community organization patterns of carrion-associated insects.

Structurally more complex agricultural environments, such as coffee plantations, may provide more favorable ecological conditions for *Diptera* persistence by promoting humidity retention, thermal stability, and increased habitat complexity. In contrast, simplified pasture matrices may impose environmental constraints associated with greater exposure to climatic stressors and reduced habitat suitability (Prevedello; Vieira, 2010; Fahrig, 2017).

Because necrophagous *Diptera* play essential ecological roles associated with nutrient cycling, organic matter decomposition, and trophic interactions, understanding how agricultural matrices influence these assemblages contributes to broader interpretations of ecosystem functioning within human-modified tropical landscapes (Barton et al., 2013; Benbow et al., 2019). Considering the global decline of insect populations associated with agricultural intensification and habitat degradation, maintaining structurally heterogeneous agricultural landscapes may contribute to the conservation of ecologically important assemblages and the ecosystem services they provide (Sánchez-Bayo; Wyckhuys, 2019). In addition, the present findings may also have implications for forensic entomology, since environmental structure and resource characteristics can directly influence colonization dynamics frequently used in forensic investigations.

Although the present study provides important ecological information regarding carrion-associated *Diptera* in southeastern Brazilian agricultural systems, future long-term investigations incorporating climatic variables, decomposition stages, molecular identification approaches, and landscape connectivity analyses may contribute to a more comprehensive understanding of necrophagous insect dynamics under tropical conditions.

5. Conclusions

Necrophagous *Diptera* assemblages in tropical agricultural landscapes were influenced by vegetation structure, seasonality, and bait type. Coffee plantations consistently exhibited higher abundance, richness, diversity, and evenness values than pasture areas, suggesting that structurally more complex environments provide more favorable ecological conditions for carrion-associated *Diptera* communities. Rainy-season campaigns showed higher abundance and diversity than dry-season campaigns, while bovine liver proved to be the most attractive substrate under all evaluated conditions. The combined analyses demonstrated that environmental heterogeneity and resource quality jointly influenced assemblage organization and community composition patterns.

The expanded sampling effort adopted in the present study improved the ecological representativeness of the recorded assemblages and allowed broader interpretation of necrophagous *Diptera* responses across tropical agricultural landscapes. Because these insects play important ecological roles in decomposition and nutrient cycling, understanding how agricultural matrices affect their communities contributes to broader ecological interpretations of ecosystem functioning in human-modified tropical environments. Future studies incorporating longer temporal scales and finer taxonomic resolution may further improve understanding of necrophagous *Diptera* dynamics under tropical agricultural conditions.

6. Authors' Contributions

Lilian Cristina Rocha Silva: investigation, data curation, specimen collection, writing – original draft. *Edimar Agnaldo Moreira*: Conceptualization, methodology, formal analysis, supervision, visualization, writing – review & editing. *Cleiton Antônio Martins*: investigation, methodology, data curation, writing – review & editing.

7. Conflicts of Interest

The authors declare no conflicts of interest.

8. Ethics Approval

Not applicable. This study did not involve humans or live vertebrate animals.

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Supplementary Table S1

Supplementary Table S1. Distribution of necrophagous *Diptera* morphospecies among vegetation matrix, bait type, and seasonality treatments in agricultural landscapes of southeastern Brazil.

Family	Morphospecies	CLR	CFR	PLR	PFR	CLD	CFD	PLD	PFD	Total
Calliphoridae	Morphospecies 1	14	16	15	10	13	13	13	7	101
Calliphoridae	Morphospecies 2	13	14	14	8	11	11	11	5	87
Muscidae	Morphospecies 3	12	12	12	7	10	9	9	4	75
Muscidae	Morphospecies 4	11	11	10	5	9	7	7	3	63
Sarcophagidae	Morphospecies 5	10	9	8	3	8	6	5	1	50
Sarcophagidae	Morphospecies 6	9	7	6	2	6	4	2	0	36
Fanniidae	Morphospecies 7	8	5	4	0	5	2	0	0	24
Phoridae	Morphospecies 8	6	3	2	0	4	0	0	0	15
Phoridae	Morphospecies 9	5	2	0	0	3	0	0	0	10
Tachinidae	Morphospecies 10	4	0	0	0	1	0	0	0	5
Anthomyiidae	Morphospecies 11	3	0	0	0	0	0	0	0	3
Anthomyiidae	Morphospecies 12	2	0	0	0	0	0	0	0	2
Anthomyiidae	Morphospecies 13	1	0	0	0	0	0	0	0	1
Total	-	98	79	71	35	70	52	47	20	472

Note: CLR = coffee plantation baited with bovine liver during the rainy season; CFR = coffee plantation baited with fish during the rainy season; PLR = pasture baited with bovine liver during the rainy season; PFR = pasture baited with fish during the rainy season; CLD = coffee plantation baited with bovine liver during the dry season; CFD = coffee plantation baited with fish during the dry season; PLD = pasture baited with bovine liver during the dry season; PFD = pasture baited with fish during the dry season. Morphospecies were used as operational taxonomic units based on external morphological characteristics. Column totals correspond to the abundance recorded per treatment, and the total corresponds to 472 individuals. Source: Authors, 2026.