





## Article

# Monitoring Stresses Caused by Gaseous Pollutants: How Can They Affect a Fruit-Feeding Butterfly Community (Lepidoptera: Nymphalidae) in the Caatinga?

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**Abstract:** This study evaluated the effects of gaseous pollutants and vegetation on the structure of fruit-feeding butterfly communities (some subfamilies of Nymphalidae) in a Caatinga area in Brumado, BA, between 2016 and 2018. Two transects were established: Transect “I” (presence of pollutant plumes) and Transect “II” (absence), encompassing a forest fragment and pasture. Bait traps were installed in each transect, and the butterfly communities were analyzed using faunistic indices, including species richness, Shannon diversity index, abundance, and dominance. The canopy opening was also assessed. The composition of fruit-feeding butterfly communities was influenced by both pollutants and vegetation. Gaseous pollutants increased butterfly abundance, diversity, and species richness, though species dominance remained unaffected. Notably, the abundance of *Hamadryas februa* was particularly sensitive to pollutant exposure. Conversely, increased canopy opening was negatively associated with butterfly abundance and diversity. A relationship between canopy opening and the presence of gaseous pollutants may reflect changes in the abundance and diversity of fruit-feeding butterfly species in the study region. Long-term community monitoring is important, as interannual differences in population fluctuations are common. A better understanding of the patterns found is essential to for devise devising conservation strategies for frugivorous butterfly communities in mining ventures.

**Keywords:** faunistic analysis; biomonitoring; air pollution stresses



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## 1. Introduction

Human activities, including industrial, urban, and agricultural practices, generate a wide array of toxic chemicals that can significantly impact the environment [1]. Biomoni-

toring, the assessment of living organisms' responses to environmental changes, provides a critical tool for evaluating these impacts [2,3]. Changes in the abundance, diversity, and composition of bioindicator groups often signal environmental stress [4]. Effective bioindicators must be overly sensitive to specific stress factors, reflect changes in ecosystem structure [5,6], belong to well-studied taxonomic groups, and display either restricted or widespread occurrence across diverse environmental conditions [7].

Insects, which comprise the majority of global biodiversity [8], are particularly well suited as bioindicators due to their rapid response to environmental stressors and broad geographical distribution [9]. Among insects, butterflies, members of the order Lepidoptera, are widely studied. They are categorized into two guilds based on adult feeding habits: nectar feeders and fruit feeders composed of some subfamilies of Nymphalidae [10,11]. While butterfly responses to ecological disturbances have been extensively studied in tropical forests [12], little is known about their sensitivity to gaseous pollutants, particularly in fruit-feeding butterfly communities near mining zones within the Caatinga biome, especially the frugivorous butterflies guild, for which comparative studies in disturbed environments and monitoring programs are increasing, due to the advantage of standardizing the sampling effort, through the use of attractive traps, which denote sampling success regardless of the collector's skill [13].

The mining company Magnesita Refratários S.A., located in Brumado, Bahia, Brazil, is the country's largest producer of magnesite ore, contributing approximately 80% of national production. The primary pollutants emitted by the company include sulfur dioxide (SO<sub>2</sub>) and nitrogen oxides (NO<sub>x</sub>) [14,15], which contribute to air pollution through suspended particles and gaseous emissions [16]. These pollutants can accumulate in the soil, degrading its quality and fertility, and may cause phytotoxic effects on plants [17], thereby potentially reducing agricultural production in surrounding areas.

Air pollution affects insect communities directly and indirectly. It can impair foraging, flight, and feeding behaviors during the larval stage, extend development time, and reduce overall insect abundance, particularly of pollinators [18]. Conversely, it can increase the density of phytophagous insects, such as sap feeders [19]. These effects are linked to changes in plant nutritional value, secondary metabolite levels, increased palatability for herbivores, and decreased predation and parasitism [20].

Changes in vegetation can further alter the composition and dynamics of animal communities, impacting crucial processes like migration, survival, and reproduction [21]. The interplay between vegetation type and gaseous pollutants remains poorly understood for local insect communities. Identifying these stressors is essential for implementing conservation measures and promoting long-term ecosystem recovery. Additionally, variations in canopy openness and light incidence can directly affect the distribution of some butterfly species [22–26].

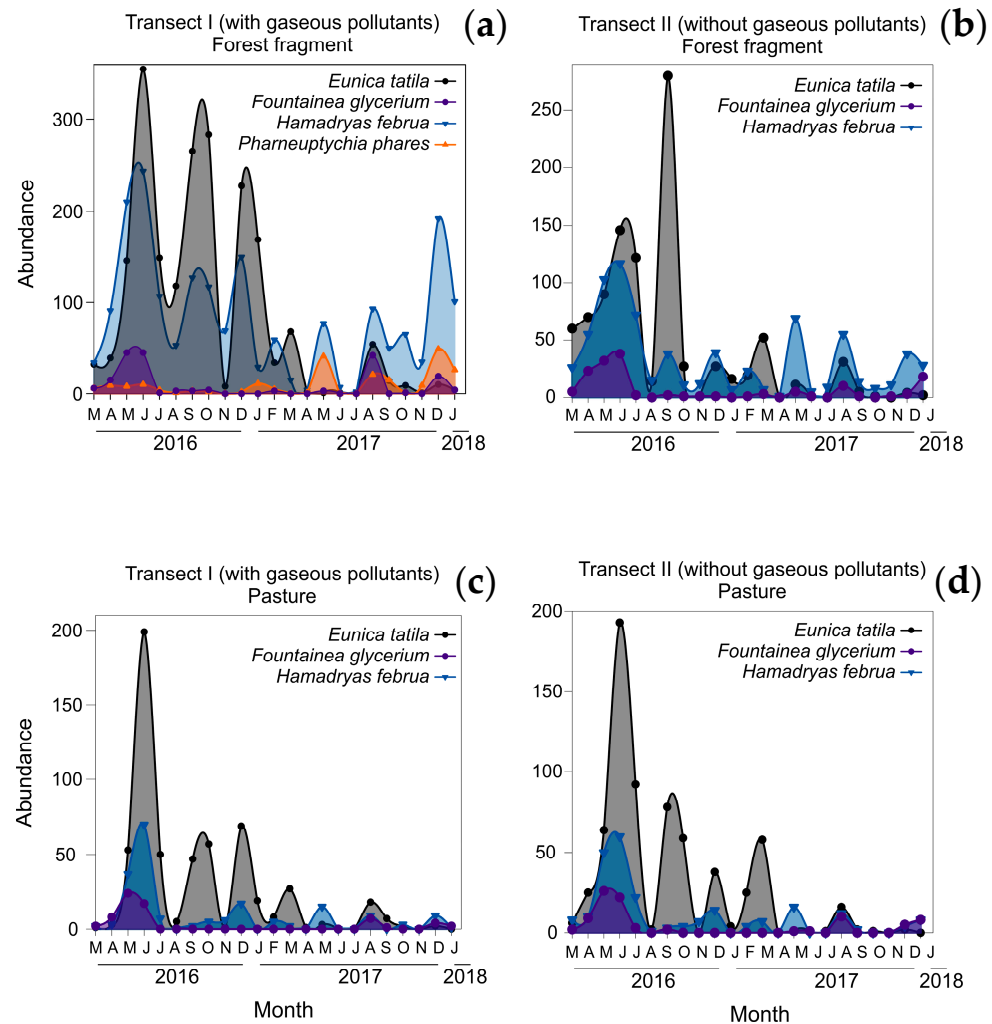
This study aimed to assess the impacts of gaseous pollutants and vegetation types on the structure of fruit-feeding butterfly communities in a mining area within the Caatinga biome.

## 2. Results

### 2.1. Community Structure and Population Dynamics of Fruit-Feeding Butterflies in the Caatinga

A total of 8481 individuals, representing 12 genera and 19 species of fruit-feeding butterflies (Nymphalidae) across four subfamilies (Biblidinae, Charaxinae, Nymphalinae, and Satyrinae), were recorded. These species were distributed among ten tribes: Ageroniini, Anaeini, Biblidini, Brassolini, Callicorini, Coeini, Ephiphilini, Epicaliini, Eurytelini, and Satyrini (Tables S1 and S2, Supplementary Material).

The species most frequently represented among the specimens collected include *Eunica tatila*, *Hamadryas februa*, *Pharneuptychia phares*, *Fountainea glycerium*, and *F. halice* (Figure 1).



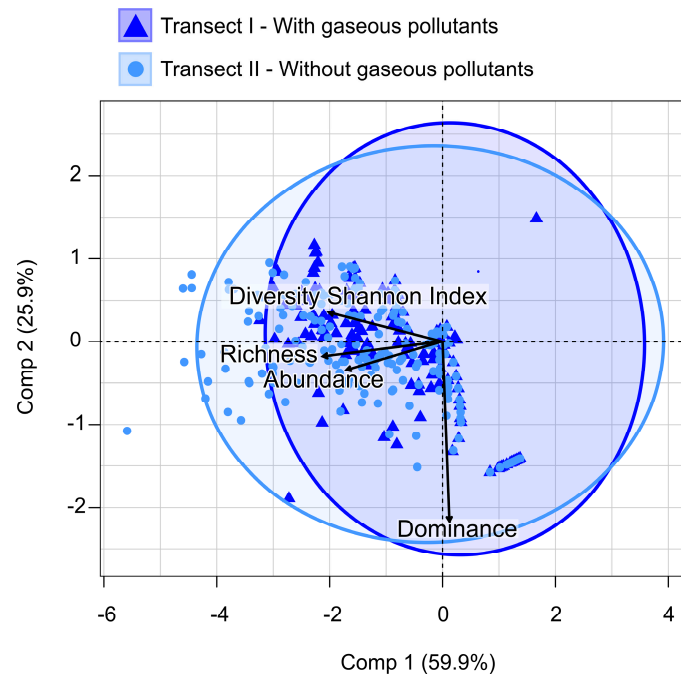
**Figure 1.** Annual fluctuation of the abundance of fruit-feeding butterflies in pasture and forest fragments within a Caatinga area during 2016 and 2018. Transect I, forest fragment (a); Transect II, forest fragment (b); Transect I, pasture (c); and Transect II, pasture (d).

Greater numbers of individuals of the most abundant species were found for *Eunica tatila* and *Hamadryas februa* sampled across both transects (Figure 1). In Transect I, the forest fragment exhibited significantly higher species abundance than the pasture area (Figure 1a,b). A similar pattern was observed in Transect II, where the forest fragment also supported a greater abundance of fruit-feeding butterflies compared to the pasture (Figure 1c,d).

## 2.2. Effects of Gaseous Pollutants on Butterfly Abundance, Diversity, Richness, and Dominance

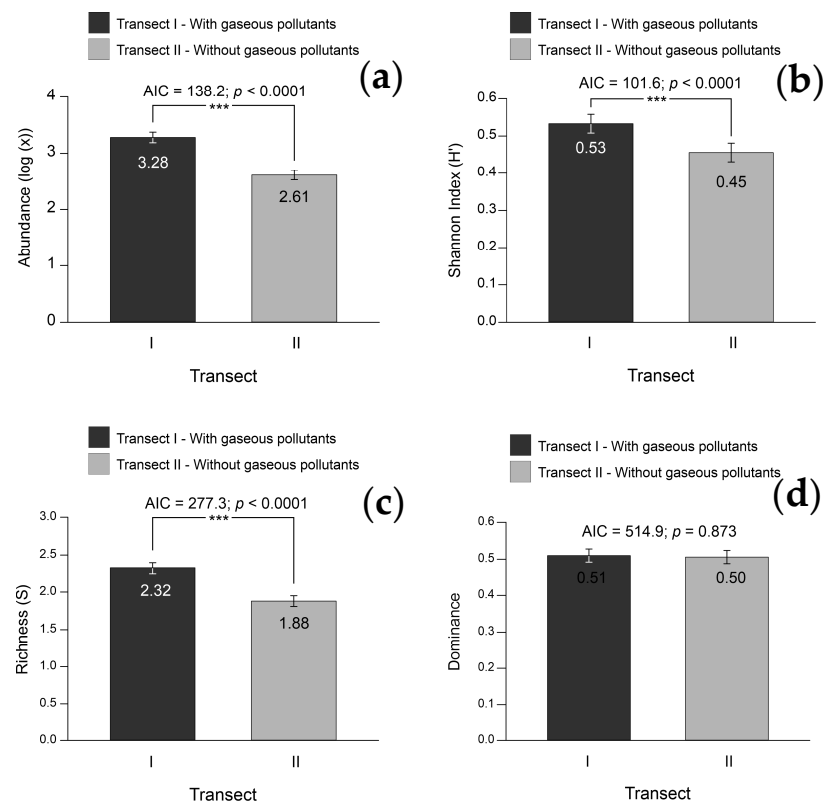
The abundance, diversity (Shannon–Wiener index  $H'$ ), species richness, and dominance of fruit-feeding butterflies followed similar patterns in Transects I and II (Figure 2).

Principal Component Analysis (PCA) indicated that 85.8% of the variation in faunal indices was explained by Components 1 and 2. Abundance and species richness showed a strong positive correlation (0.83), with a moderate correlation (0.50) with diversity (Shannon–Wiener  $H'$ ) (Figure 2), while dominance exhibited weak positive correlations with abundance (0.15) and richness (0.14) and a weak negative correlation with diversity (Shannon–Wiener index  $H'$ ) (−0.18).



**Figure 2.** Principal Component Analysis (PCA) for abundance, diversity (Shannon–Wiener index  $H'$ ), species richness, and dominance of fruit-feeding butterflies in a Caatinga area with (Transect I) and without (Transect II) gaseous pollutants.

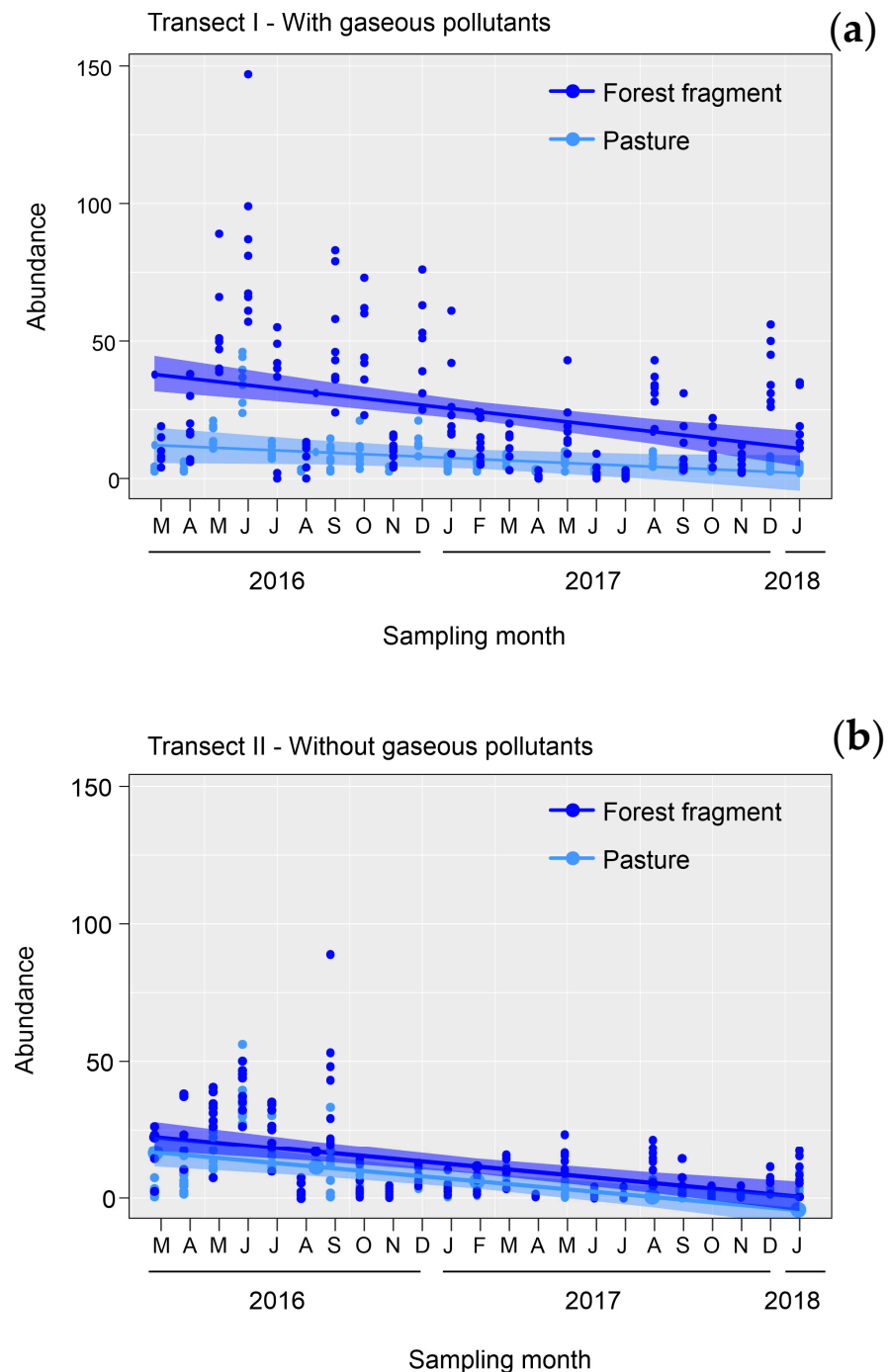
Generalized linear models revealed significant differences in abundance ( $p < 0.0001$ ), diversity (Shannon–Wiener index  $H'$ ) ( $p < 0.0001$ ), and species richness ( $p < 0.0001$ ) between Transects I and II (Figure 3).



**Figure 3.** Comparison of abundance (a), diversity (Shannon–Wiener index  $H'$ ) (b), species richness (c), and dominance (d) of fruit-feeding butterfly species in areas with and without exposure to gaseous pollutants in the Caatinga.

Species abundance (Figure 3a), diversity (Figure 3b), and richness (Figure 3c) were higher in Transect I, which was influenced by gaseous pollutants, compared to Transect II. No significant differences in species dominance ( $p = 0.873$ ) were observed between the transects (Figure 3d).

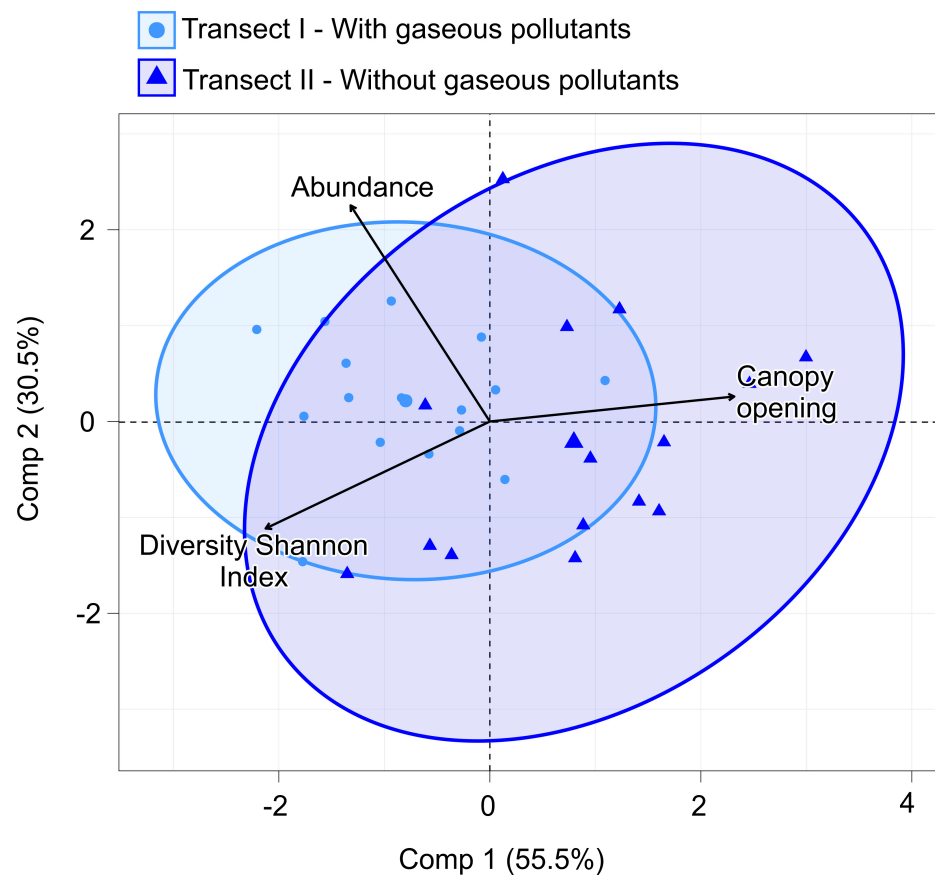
A negative linear relationship was identified between species abundance and sampling time for both transects (Figure 4). Abundance decreased as the time elapsed between sampling events increased.



**Figure 4.** Changes in butterfly species abundance from 2016 to 2018 in Caatinga areas with (Transect I) (a) and without (Transect II) (b) exposure to gaseous pollutants. LMER with Akaike's Information Criterion for small samples ( $\Delta AIC: 1866.15$ ) was used to select the optimal model ( $R^2 = 0.70$ ).

### 2.3. Canopy Openness and Its Relationship with Abundance and Diversity of Fruit-Feeding Butterfly Species

Principal Component Analysis (PCA) showed a correlation between canopy openness, species abundance, and diversity (Shannon–Wiener index  $H'$ ) (Figure 5). Components 1 and 2 explained approximately 86% of the total variation. A weak positive correlation (0.21) was found between abundance and diversity, while a weak negative correlation was observed between canopy openness and both abundance and diversity (Shannon–Wiener index  $H'$ ) ( $-0.14$ ).



**Figure 5.** Principal Component Analysis (PCA) showing the relationship among species abundance, species diversity (Shannon–Wiener index  $H'$ ), and canopy openness in a Caatinga area with and without exposure to gaseous pollutants.

### 3. Discussion

All fruit-feeding butterfly subfamilies recorded in this study have previously been documented in Brazil's semiarid region, including Bahia [27–30]. Biblidinae species, in particular, benefit from forest fragmentation, as early-stage regeneration areas offer an abundance of host plants for this group [31]. The specific characteristics of Caatinga vegetation, such as its floristic composition, climate, and canopy structure, representing a less structurally complex environment than tropical rainforests [27,32,33], may justify the greater representativeness of Biblidinae in this phytogeographic domain.

Among the *Biblidinae*, the tribes *Epicalini* and *Ageroniini* were the most abundant, with *Eunica tatila* and *Hamadryas februa* being the dominant species across both transects and vegetation types. Similar trends were reported by Beirão et al. [34] in a transitional Cerrado–Caatinga region in northern Minas Gerais, where these species accounted for approximately 84% of the community. Their abundance in the Caatinga is likely linked to the availability of host plants for caterpillars [33] and their adaptability to open habitats

and semi-deciduous forests [34,35]. While little is known about the biology of *Eunica tatila*, its prevalence in semi-deciduous forests is well established [34]. For *H. februa*, its distribution closely aligns with that of its host plants, as butterflies are often highly specific to larval food resources, which determine their local distribution [35,36]. However, little is known about the dietary specificity of some fruit-feeding butterfly species, especially in the Caatinga.

Butterfly abundance varied significantly between transects and vegetation types, with the overall abundance pattern primarily driven by *E. tatila* and *H. februa*, which accounted for 88.04% of the total population. Studies suggest that species abundance and richness are often higher in disturbed environments compared to preserved ones [31], a trend influenced by specific environmental factors [31].

The high abundance of butterflies in Transect I can likely be attributed to the presence of the pollutant plume, predominantly composed of sulfur dioxide and nitrogen oxides. Other studies indicate that such pollutants may increase phytophagous insect densities by altering plant nutritional values, secondary metabolite levels, and plant palatability, while simultaneously reducing predation and parasitism pressures [19].

The pollutant plume in Transect I may also have favored the immature stages of fruit-feeding butterflies. However, confirming this hypothesis requires sampling caterpillars on host plants within both transects. Additionally, slight variations in the composition and availability of food resources between the transects could also influence butterfly abundance.

The diversity of fruit-feeding butterflies exhibits variation within the same biome. Previous studies conducted in regions characterized by a semi-arid climate and predominantly Caatinga vegetation have documented 15 species [28], 28 species [37], and 22 species [38] of fruit-feeding butterflies. The diversity observed in this study was generally lower than that reported in other studies on fruit-feeding butterflies using similar sampling methods [39–41]. According to Thomazini and Thomazini [7], disturbed areas resulting from deforestation, forest fragmentation, or pasture formation often experience a reduction in insect species richness and diversity. However, in some cases, such disturbances can lead to increased local diversity. As a result, no definitive generalizations can be made regarding the impact of disturbances on insect communities.

The lower diversity of fruit-feeding butterflies in pasture environments compared to forest fragments in this study may be attributed to differences in microclimatic conditions, habitat heterogeneity, and host plant diversity. Forest fragments likely provide a more humid microclimate, greater structural complexity, and a wider variety of host plants than pastures.

Variations in canopy openness between forest environments affect light incidence and may have also influenced butterfly communities. The forest fragment in Transect I (with a pollutant plume) exhibited the lowest light incidence and the highest abundance of fruit-feeding butterflies compared to Transect II (without the pollutant plume). Differences in light availability, influenced by canopy openings, likely contributed to the variation in butterfly abundance between transects.

Canopy openness can significantly impact butterfly distribution, as some species prefer shaded environments while others thrive in open, sunlit areas [21]. For instance, Santos et al. [42] note that certain fruit-feeding butterflies avoid high light exposure, favoring shaded areas, while others are more active in sunlit environments such as forest edges or natural clearings.

Given that these butterflies, particularly *H. februa*, are well adapted to open environments such as forest edges, clearings, sunlit areas [35,43,44], and regeneration habitats [23], several important questions arise: Does the pollutant plume favor the immature stages

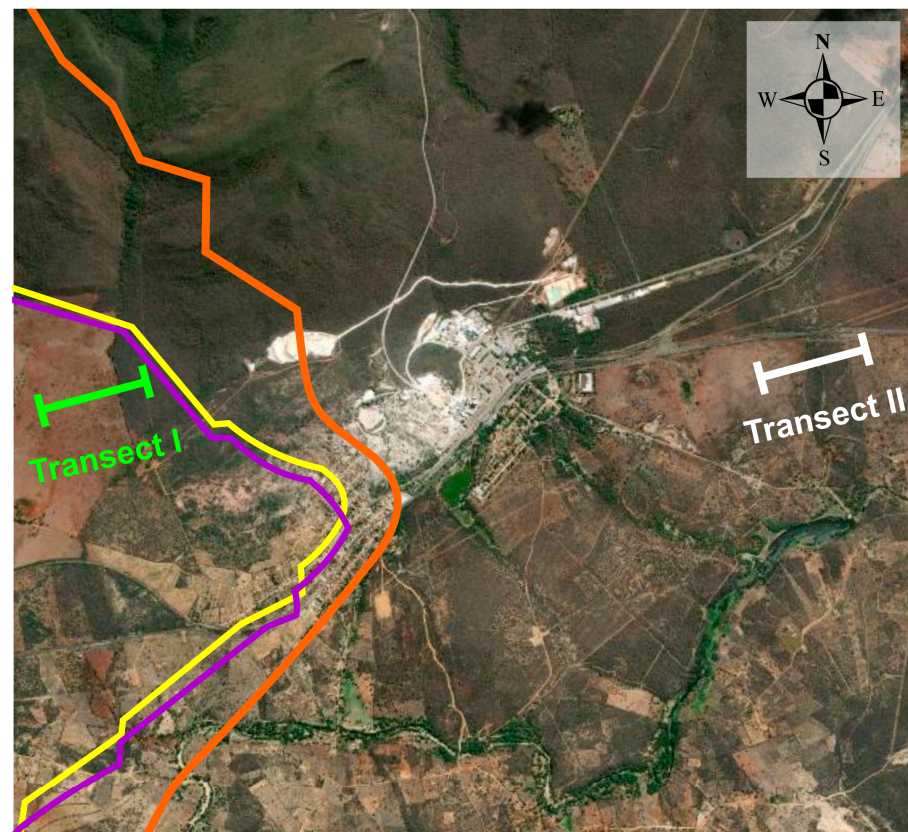
of these species? What host plants are utilized by their caterpillars? Are there significant differences in food resource availability between Transects I and II?

## 4. Materials and Methods

### 4.1. Experimental Period and Site

The study was conducted from March 2016 to January 2018 near the Catiboaba industrial unit of the Magnesita Refratários S.A. mining company, in Brumado, southwestern Bahia State, Brazil ( $-14^{\circ}12' S$ ;  $-41^{\circ}39' W$ ; and 422 m altitude ASL) (Figure 6).

- Boundaries of plume dispersion of  $\text{NO}_x$
- Boundaries of plume dispersion of  $\text{SO}_2$
- Boundaries of plume dispersion of particulate material



SYSTEM OF COORDINATES (UTM):  
SIRGAS 2000; MC 39°GrW

0 0.45 0.9 1.35  
Kilometers

**Figure 6.** Location of the transects for fruit-feeding butterfly (Nymphalidae) sampling in the industrial area of the Magnesita Refratários S.A. company.

The municipality of Brumado is located within the Caatinga phytogeographic domain, characterized by a semi-arid (hot and dry) climate classified as BSh according to the Köppen-Geiger system [45]. The rainy season occurs from October to March, with annual rainfall ranging from 229 to 1118 mm. The rest of the year is marked by scarce rainfall, with an average of 640 mm and a mean temperature of  $23.8^{\circ}\text{C}$  [46].

The study sites were selected based on a 2009 dispersion report on pollutant plumes [13], which remains the only evaluation of pollutant concentration and dispersion for the area to date. According to this report, the maximum daily concentration of sulfur dioxide ( $\text{SO}_2$ ) was  $6.9 \mu\text{g m}^{-3}$ , recorded approximately 1500 m west-southwest (W-SW) of the mining company, while the annual maximum was  $1.38 \mu\text{m}^{-3}$  at around



3900 m in the same direction. For nitrogen oxides ( $\text{NO}_x$ ), the maximum daily concentration reached  $4353.9 \mu\text{m}^{-3}$  approximately 7400 m south-southwest (S-SW), with an annual maximum of  $89.14 \mu\text{m}^{-3}$  recorded about 3900 m west-southwest (W-SW) of the mining company [13]. The *Fabaceae*, *Euphorbiaceae*, and *Burseraceae* families are the most prevalent. In the pasture area, the herd consists of domestic cattle. The cattle farming industry in the area surrounding the magnesite mining company is extensive and predominantly family-owned. The predominant breed is the Nelore.

#### 4.2. Sampling and Analysis of Fruit-Feeding Butterfly Communities

For sampling fruit-feeding butterflies, Van Someren–Rydon traps (Log Nature, Belo Horizonte, MG, Brazil) [47,48] were used. These traps were mounted on wooden tripods 1.80 m high, permanently fixed to the ground, with the trap itself positioned one meter above ground level [47,49]. The traps were installed along two transects, each measuring 850 m in length and 5 m in width, in the following areas: Transect I: located in an area exposed to pollutant plumes from industrial activity, approximately 2 km from the ore extraction and processing site ( $-14^\circ 14' 37''$  S,  $-41^\circ 44' 40''$  W), and Transect II: located in an area free from pollutant plumes, approximately 4 km from industrial activity ( $-14^\circ 13' 36''$  S,  $-41^\circ 43' 06''$  W). The two transects were separated by an approximate distance of 3.5 km (Figure 6).

Both transects traversed areas of forest fragments composed of medium and small-sized trees, xerophilous shrubs typical of the Caatinga biome, and pasturelands. These sites were georeferenced using UTM coordinates (SIRGAS 2000, Zone 24 L) with a Garmin Etrex Vista® H GPS device (Garmin®, Kansas City, MO, USA). Each transect included 16 collection points spaced 50 m apart within each vegetation type (eight in forest fragments and eight in pastures), with a total of 32 collection points. The points were spaced 125 m apart and were sampled monthly.

Traps were baited with 0.25 L of a mixture prepared with 2 kg of overripe bananas, 0.25 kg of brown sugar, and 0.5 L of water heated to  $65^\circ\text{C}$ . The bait was prepared 30 h before sampling [13,49,50]. Traps were exposed in the field for 24 h, after which captured butterflies were manually removed, euthanized by gently pressing the thorax, and placed in entomological envelopes.

Specimens were sent to the Laboratory of Entomology at the Universidade Estadual do Sudoeste da Bahia, Campus Vitória da Conquista, for identification to the species or subspecies level. The captured specimens were identified to the species level by specialist Dr. Laura Braga, using identification guides, such as those of Jekins [35], Uehara et al. [50], Freitas [13], Barbosa et al. [51–53], Zacca et al. [54], Dias et al. [55], Gueratto et al. [56], and Palo Jr. [57], when possible, according to the taxonomic nomenclature of Lamas [58].

#### 4.3. Forest Fragment Canopy Estimation

Luminosity patterns within the forest fragments of both transects were measured at the locations of the eight fruit-feeding butterfly traps during the dry season (September 2017) and the wet season (December 2017). These measurements were conducted using a forest densiometer (Spherical Densiometer Model—A, Forest Densiometers, Rapid City, SD, USA) to estimate the percentage of canopy openings in the forest environment [59].

#### 4.4. Statistical Analysis

Faunistic indices, including abundance (number of individuals), richness (number of species), dominance (a species is considered to be dominant when its relative frequency exceeds  $[1/S] \times 100$ , where  $S$  denotes the total number of species within the community), and the Shannon–Wiener diversity index (Shannon–Wiener  $H'$ ). The Shannon–Wiener index ( $H'$ ) is calculated as a function of the number of species and the proportion of

individuals belonging to a given species in a community [60]. All the faunistics indices were calculated using the “vegan” package [61]. To explore correlations and associations between variables across the two transects (I: with pollutants; II: without pollutants), a Principal Component Analysis (PCA) was performed using the “factoextra” package [62].

To assess the effects of gaseous pollutants on the abundance of fruit-feeding butterfly communities, generalized linear models (GLMs) were constructed with a significance level of  $p < 0.05$ . Richness and abundance data were modeled using a Poisson distribution with a log link, while Gaussian distribution models were applied for diversity (Shannon–Wiener  $H'$ ) and dominance indices, utilizing the “lme4” [63] and “lsmeans” [64] packages.

A linear mixed-effects model was developed to analyze the effects of these stressors over time. Fixed factors in the model included transect (I or II) and vegetation type (pasture or forest fragment), while collection traps were treated as a random effect.

Additionally, PCA was used to identify correlations between canopy openness and the abundance and diversity of fruit-feeding butterfly species. All statistical analyses were conducted using R statistical software, version 4.3.1 [65].

## 5. Conclusions

A relationship between canopy opening and the presence of gaseous pollutants may reflect changes in the abundance and diversity of fruit-feeding butterfly species in the study region.

The results of the present study illustrate a possible effect of gaseous emissions on the structure of the frugivorous butterfly community in the Caatinga region. However, many factors may be associated with the patterns found, such as the floristic composition and microclimatic characteristics of each area (under the effect of the plume and without the effect of the plume). Therefore, future studies are needed to answer the questions raised by the results, especially concerning the caterpillar stage.

Long-term community monitoring is important, as interannual differences in population fluctuations are common. A better understanding of the patterns found is essential for devising conservation strategies for frugivorous butterfly communities in mining ventures.

**Supplementary Materials:** The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/stresses5010003/s1>, Table S1. Abundance (No.) and relative frequency (Fr%) of fruit-feeding butterfly species and subspecies (Nymphalidae), as a function of subfamilies and tribes. Table S2. Faunistic indexes of fruit-feeding butterfly species (Nymphalidae) according to transects I (with pollutant plume) and II (without pollutant plume) and vegetation types (forest fragment and pasture).

**Author Contributions:** Conceptualization, G.d.S.S., M.A.C. and L.B.; data curation, G.d.S.S., M.A.C., D.R.d.C., M.d.S.d.S., E.C.d.M. and W.G.O.S.; formal analysis, G.d.S.S., R.J.d.S.N., A.d.O.S.F. and M.P.d.S.; funding acquisition, M.A.C.; investigation, G.d.S.S., M.A.C., M.d.S.d.S., E.C.d.M., W.G.O.S. and D.R.d.C.; methodology, G.d.S.S., M.A.C., L.B., M.d.S.d.S. and A.d.O.S.F.; project administration, M.A.C.; resources, G.d.S.S., M.A.C., M.d.S.d.S., E.C.d.M., W.G.O.S. and D.R.d.C.; software, G.d.S.S., R.J.d.S.N., E.C.d.M. and M.P.d.S.; supervision, M.A.C.; validation, G.d.S.S., M.A.C., D.R.d.C., L.B. and A.d.O.S.F.; visualization, G.d.S.S. and M.A.C.; writing—original draft, G.d.S.S. and M.A.C.; writing—review and editing, M.A.C., S.A.L., L.B., R.J.d.S.N. and M.P.d.S. All authors have read and agreed to the published version of the manuscript.

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**Data Availability Statement:** The data that support the findings of this study are available from the corresponding author upon formal request.

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**Conflicts of Interest:** The authors declare that there are no conflicts of interest.

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