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Interaction of Filth Flies and Epigeal Arthropods with Soil Nitrogen and Gas Emissions in Grazing Systems under a Legacy of Low Fertilization

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Abstract: During the summers of 2021 and 2022, we conducted a study in four Georgia Piedmont pastures to assess the effect of the presence of filth flies and epigeal arthropods on carbon and nitrogen emissions and soil nitrogen retention from lax rotational grazing systems under a legacy of low fertilization. Carbon dioxide (CO_2), nitrous oxide (N_2O), and ammonia (NH_3) emissions were measured from dung on days 0, 4, 8, and 15 following depositions. Soil and manure samples were collected on days 0 and 16 and analyzed for ammonium (NH_4^+), nitrate (NO_3^-), plant-available nitrogen (PAN), and potentially mineralizable nitrogen (PMN). Manure samples were analyzed for total Kjeldahl nitrogen (TKN). The numbers of filth flies ovipositing and emerging from manure, fire ants, and epigeal arthropods around the manure were determined. Our results indicated that more than 12 ovipositing filth flies per manure pat can reduce PMN by up to 14.7 kg of nitrogen per hectare, while an increase in the biodiversity and abundance of predators may help to increase PAN and PMN in grazing systems, as well as decrease the number of emerging filth flies.

Keywords: nitrous oxide emissions; ammonia volatilization; carbon dioxide emissions; filth flies; fire ants; cattle manure; grazing systems

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1. Introduction

Pastures constitute more than 25% of global land use and around 60% of agricultural land in the United States [1,2]. Global meat cattle production has more than doubled since 1961, and the US is the main meat producer [3]. Cattle production is one of the most important agricultural industries in the US, and in 2022 it represented 17% of the total cash receipts for agricultural commodities [4]. Grazing systems must be managed carefully because they protect key regulating ecosystem services such as carbon storage, biological control, pollination, and water supply [5–7] but also because unsustainable practices may lead to an increase in greenhouse gas emissions, a severe pollution of water systems, and soil degradation [8–10].

Small-scale farms, with sales values of less than $100,000 per year hold 31% of the U.S. cattle inventory, and the production of beef cattle is their main agricultural activity [11]. In the state of Georgia, 83% of cattle farms are small farms that earn less than $150,000 annually [12]. About 87% of small farms depend on family labor, and more than 50% of beef farm operators are engaged in other activities as their primary occupation [13,14]. As a result, small farms typically exhibit less rigorous management practices and have reported that forage availability and external parasites had a significant economic impact on their cattle operations [14].

Nitrogen (N) is almost always the most limiting nutrient for grass production on sandy and sandy clay, acid, low-organic-matter, and low-fertility soils of Georgia, USA. These soil conditions, accompanied by the high temperatures and humid conditions of Georgia summers can lead to N loss as emissions from cattle manure [15,16].

Filth flies are a common nuisance for cattle on pasture; they oviposit in cattle manure, and the larvae consume bacteria and organic matter until pupation [17,18]. High infestation numbers—especially of horn flies (*Haematobia irritans* Linnaeus), which are hematophagous cattle ectoparasites and can reduce the productivity and profitability of grazing systems [19–21]. Besides economic and health issues induced by filth flies, N emissions from cattle manure have been reported in the presence of house flies (*Musca domestica* Linnaeus) and bottle flies (*Lucilia sericata* Meigen) [22–24]. In addition, epigeal arthropods such as dung beetles, herbivorous insects, and red imported fire ants (*Solenopsis invicta* Buren) have also been associated with intervening in soil N retention and emissions [25–28]. Furthermore, red imported fire ants, rove beetles, and other members of the epigeal arthropod community have been shown to decrease filth fly numbers emerging from bovine manure [29,30].

Abiotic factors also have a role in emissions. Ammonia (NH_3) emissions can increase by 48% when the temperature changes from 20 to 30 °C, and increase from undetectable levels to 9.8% of the initial total N of stable manure when the pH changes from 7.5 to 9 [31]. Ammonia emissions from cattle manure doubled when the pasture received 11.3 mm, rather than 0 mm, of cumulative rainfall during the 4 days before sampling [32]. Increases in temperature from 20 to 40 °C and in manure moisture content from 65 to 75% have been shown to significantly increase carbon dioxide (CO_2) emissions [33]. Nitrous oxide (N_2O) emissions can increase more than ten-fold when temperatures are higher than 10 °C—the maximum temperature reported was 25 °C—but only when the soil moisture is near field capacity [34]. Rainy seasons can promote the generation of N_2O since rain enters the water-filled pore space (WFPS) and limit O_2 diffusion, initializing the denitrification processes when the WFPS is >0.6 [16,35]. An increase in porosity in the manure can also result in higher N emissions [36,37].

Because NH_3 emissions are greater from cattle urine than from cattle manure most work has looked at urine N emissions in grazing systems, resulting in a knowledge gap on N emissions from manures, especially in terms of filth flies, fly predator, and the provisioning of soil inorganic N (plant available N). Considering that there may be several trophic and abiotic interactions that influence filth fly numbers and N losses in grazing systems, this paper aimed to assess the effect of the presence of filth flies and soil arthropods on C and N emissions and soil N retention in lax rotational grazing systems under low-fertilization-legacy conditions in the Georgia Piedmont, USA.

2. Materials and Methods

2.1. Characteristics of the Study Site

This study was conducted in four pastures within the Georgia piedmont; two family farms (33.782729° N, 83.330256° W; elevation 213–259 m, Watkinsville) in Oconee County, Georgia, USA, and two pastures at the Eatonton Beef Research Unit of the University of Georgia (33.420759° N, 83.476555° W, elevation 152–177 m, Eatonton) located in Putnam County, Georgia, USA. All pastures under study were grazed under a lax rotational system according to forage availability, with no fertilization practices for the last 15 years. Hay was fed and watering stations supplied by deep wells were at upland locations. Shade for beef cattle was provided in the form of trees primarily in upland positions distributed throughout different paddocks within the family farms. In the Eatonton pastures, most of the trees were upland, but some surrounded a creek in the bottomland. These pastures were declared to have a low fertilization legacy because they had not received any kind of fertilization during the last 15 years.

The soil of the Watkinsville farm pastures was mapped as Pacolet (fine, kaolinitic, thermic Typic Kanhapludults) sandy clay loam, with two slope ranges (6 to 10% and 10 to 15%) and was classified as severely eroded and well-drained. Eatonton pasture soils were mapped as Wilkes (loamy, mixed, active, thermic, shallow Typic Hapludalfs) sandy loam, with two ranges of slope (2 to 10% and 10 to 25%) and were eroded and well drained. Smaller areas were mapped as Davidson loam, with 2 to 6% slopes, moderately eroded, and well drained [38].

The monthly precipitation and average monthly temperature for Eatonton pastures and Watkinsville pastures for 2021 and 2022 (Figure 1) were obtained from the network of weather stations maintained by the College of Agricultural and Environmental Sciences, University of Georgia [39]. To calculate the total precipitation for each chamber in our findings, we summed the rainfall from the previous day and the day of sampling for each sampling day and then aggregated these values, as shown in the formula:

$$\text{Total precipitation}_{i+j} = \sum P_i + P_j, \tag{1}$$

where P_i is precipitation received on the day before sampling, and P_j is precipitation received on the sampling day. Sampling days (j) were 0, 4, 8, and 15.

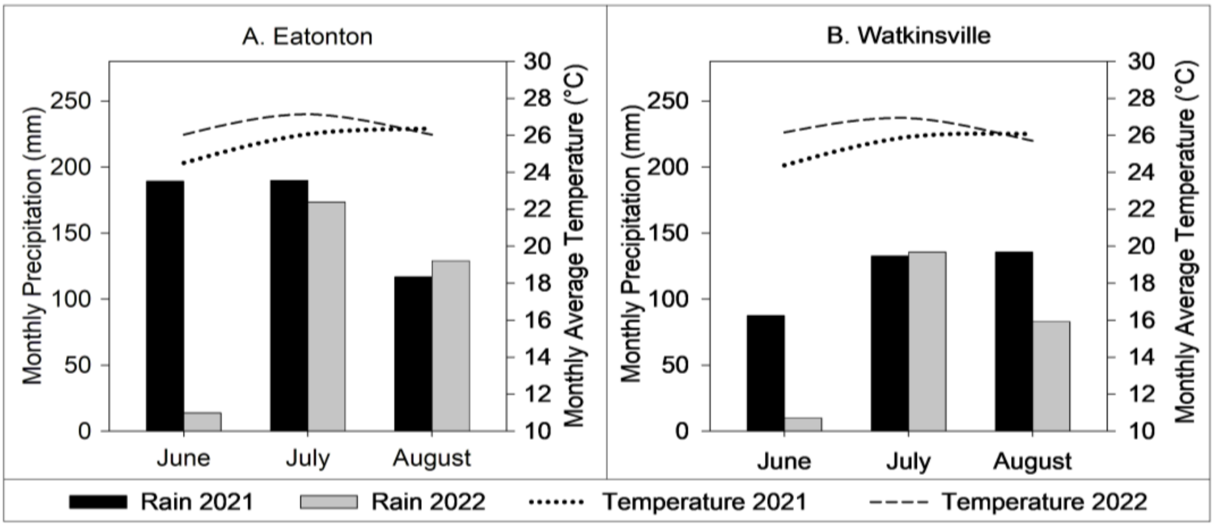

Figure 1. Monthly precipitation and average monthly temperature for 2021 and 2022 for (**A**) Eatonton and (**B**) Watkinsville.

2.2. Experimental Design and Sampling

The sampling period was from June to August in both 2021 and 2022. Each month two pastures, one from Watkinsville and one from Eatonton were sampled on the same dates. Four manure pats were sampled per pasture along with a control. A plastic chamber was installed above each manure pat and on the pasture as a control, to measure NH_3, N_2O, and CO_2 emissions for 16 days after manure pat deposition. Manure temperature (Digital Soil Thermometer Rapitest model 1625, Luster Leaf, Atlanta, GA, USA) and emissions were measured on pat deposition day 0 and days 4, 8, and 15 after deposition. On days 0 and 16, one cup of manure was sampled and frozen to determine moisture and total Kjeldahl nitrogen (TKN) content. On the same days (0 and 16), soils were sampled from four locations around each chamber at 0 to 5 and 5 to 10 cm depth to determine nitrate (NO_3^-), ammonium (NH_4^+), plant available nitrogen (PAN), and potentially mineralizable nitrogen (PMN). Filth flies ovipositing in manure on day 0, and the emerging filth flies on days 15 and 16 were captured using a net trap. On day 16, the remaining pat was collected to determine the pupal number. A pitfall trap was installed around each chamber during the deposition day to assess the abundance and type of epigeal arthropods visiting nearby chambers. Pitfall trap contents were collected on days 1, 4, 8, and 15 and analyzed to determine biodiversity by the Shannon index and to determine arthropods associated with manure pats.

2.3. Characterization of Filth Fly Populations

On the deposition day, a net trap was placed over the manure pat 1 min after deposition to collect the ovipositing filth flies (Figure 2A). The net traps remained in place over the manure for 10 min, then the net trap was covered with a black cloth for another 10 min to block the sunlight and stimulate the captured flies to move into the jar for subsequent collection (Figure 2B). Collected flies were identified and counted.

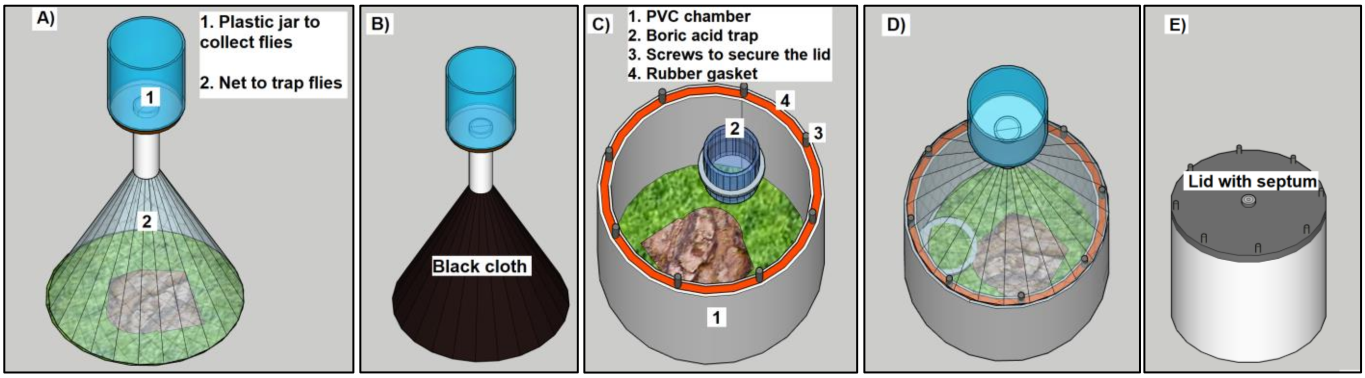

Figure 2. (**A**) Net trap on manure. (**B**) Net trap with black cloth to block sunlight. (**C**) Opened gas chamber with boric acid trap. (**D**) Open gas chamber with the net trap. (**E**) Closed gas chamber.

During the 16 days of the sampling period, the chambers were covered with net traps to avoid additional oviposition and to collect any emerging flies (Figure 2D), except for the sampling days in which the chamber was closed (Figure 2E). On the 16th day, after the final collection of emerged flies, the chambers were removed, and the manure pats were collected in plastic bags. The remaining pupae from manure pats were counted and examined using a water flotation technique [40].

2.4. Characterization of Biodiversity Populations

A circular pitfall trap was installed on the deposition day around each manure pat to evaluate the activity of epigeal arthropods (beetles, spiders, crickets, e.g.) on pastures. A 100 cm^3 open-top plastic jar was buried until the rim was level with the soil surface and filled with soapy water to half its capacity [41]. The pitfall trap contents were registered as "captured" and emptied every sampling day: 1, 4, 8, and 15 after deposition.

Ant foraging activity was measured by a modified "hot dog test" [42], which measures the number of ants that are found on a hot dog piece. On the 4th day after deposition, a 5 cm hot dog piece was placed one foot away from the manure pat in a random direction. After 25 min a picture was taken to count the ants and measure the hot dog area using IC Measure software (ImagingSource®, Version 2.0.0.286, The Imaging Source LLC., Charlotte, NC, USA, 1991–2023). The results are expressed as the number of ants per square centimeter of hot dog.

Biodiversity was calculated for each chamber using the Shannon index [43], but instead of considering species, the captured invertebrates were classified into 6 categories according to their functional types: filth flies (horn flies, bottle flies, eye gnats, face flies), dung beetles (dung beetles, rainbow scarabs), predators (spiders, paper wasps, tiger beetles, rove beetles, ants), herbivores (crickets, grasshoppers, Colorado potato beetles), fly parasitic wasps, and seed parasitic wasps. The Shannon index was calculated using the following formula:

$$\text{Shannon index (H)} = -\sum_{i=1}^{s} p_i \times \ln p_i, \quad (2)$$

where s is the number of categories (6), p is the proportion (n/N) of individuals of one category (n) divided by the total number of individuals found (N), ln is the natural log, and Σ is the sum of the calculations. In the case of ants, they were considered as the number of ants per square centimeter of hot dog.

2.5. Ammonia Volatilization, Nitrous Oxide, and Carbon Dioxide Emissions

Ammonia, N_2O, and CO_2 emissions were measured from each manure pat beginning on days 0, 4, 8, and 15. Four manure pats were sampled, and one control chamber was used per pasture. In each manure pat, a gas trap chamber (30 cm ID PVC pipe open at the bottom containing one metal ring inside with an airtight removable lid that has a septum at the top), was utilized on each sampling date to capture N_2O, CO_2, and NH_3 emissions (Figure 2C).

Within each of the chambers, the metal ring held a glass jar with boric acid (100 mL 0.4 M H_3BO_3) to trap NH_3. After the collection of flies, the chambers were inserted 5 cm into the soil, boric acid traps were placed into the metal rings and the chamber was closed for 24 h. After 24 h the jars were collected and brought back to the lab for chemical analysis. N_2O and CO_2 were measured by taking a 3 mL gas sample with a syringe and analyzed in a Varian Star 3600 CX gas chromatograph using an electron capture detector (ECD) and a thermal conductivity detector (TCD). Ammonia was measured by the change in pH of the H_3BO_3 in the glass jars followed by back titration with a weak sulfuric acid solution (0.0044 N H_2SO_4) to the initial pH of 0.4 M H_3BO_3 [44].

Emissions from control chambers were subtracted from manure chambers for those chambers in which the manure did not cover all the chamber area (Equation (3)). The area covered by manure was measured using IC Measure software on the pictures of the chambers that were taken on the deposition day.

$$\text{Manure emission} = \text{Chamber emission} - \left(\frac{\text{Soil emission} \times (\text{Chamber area} - \text{Manure area})}{\text{Chamber area}}\right). \quad (3)$$

Cumulative gas emissions were calculated by a rectangular integration of the area under the curve created by the daily emissions (Equation (4)).

$$\text{Cumulative gas emission} = \text{vd0} + \tfrac{\text{vd0}+\text{vd4}}{2} \times 4 + \tfrac{\text{vd4}+\text{vd8}}{2} \times 4 + \tfrac{\text{vd8}+\text{vd15}}{2} \times 7, \quad (4)$$

where vdi is the daily value for each sampled day (i = 0, 4, 8, 15 days after deposition).

The ammonia volatilization values were corrected using the capacity of the trap (Table 1). To evaluate the capacity of the trap, a laboratory experiment was designed. A solution of NH_4Cl (0.003 M) with a pH value of 9 was prepared and enclosed in a desiccator (9.1 L) together with an acid trap (100 mL of 0.4 M H_3BO_3) in a beaker. After 24 h, a 20 mL gas sample was taken with a syringe through a septum and was slowly injected into a fresh acid trap solution to capture the NH_3 remaining in the air. Then, the desiccator was opened, both acid traps were back-titrated, and the captured NH_3 was calculated. The NH_3 captured in the fresh acid trap served to calculate the NH_3 remaining in the desiccator:

$$NH_3 \text{ remaining} = NH_3\text{captured in fresh trap} \times \frac{9.1\text{L}}{0.002\text{L}}. \quad (5)$$

Table 1. Results from the trap capacity experiment.

Replication	NH_3 Captured with 24 h Trap (mg N)	NH_3 Captured with Fresh Trap (mg N)	NH_3 Remaining in Desiccator (mg N)	Trap Capacity (%)
1	20.6	0.08	38.4	34.9%
2	18.1	0.07	33.8	34.9%
3	18.9	0.09	40.1	32.0%
4	20.7	0.09	38.8	34.7%
Mean ± STD	19.6 ± 1.1	0.08 ± 0.01	37.8 ± 2.4	34.1 ± 1.2%

The capacity of the trap (CT) was calculated with Equation (6). The experiment was run four times and the results are shown in Table 1.

$$\text{CT} = \left(\frac{\text{mgNH}_3\text{captured with24h trap}}{\text{mgNH}_3\text{captured with24h trap} + \text{mgNH}_3\text{remaining in desiccator}}\right) \times 100\%. \quad (6)$$

2.6. Analysis of Soil and Manure Samples

After collection, soil samples were placed in sealed plastic bags. Field soil moisture content was determined with 1 g of soil dried at 105 °C for 24 h in a Precision oven (model 51221129, Jouan Inc, Winchester, VA, USA). After a field moisture analysis, samples were left to air dry for two weeks. Air-dried soil was ground and sieved (<2 mm mesh), and the four replicates per chamber were combined. Combined samples were corrected for moisture, before being stored in airtight plastic bags for a subsequent analysis at the Franklin's Sustainable Lab. Subsequently, 3 g of soil sample was extracted with 20 mL of a 2 M KCl solution (cold extraction) [45], and the colorimetric measurement of ammonium (NH_4^+-N) and nitrate (NO_3^--N) was carried out using the Doane and Horwath method [46,47]. Plant-available nitrogen (PAN) was calculated by adding NH_4^+-N and NO_3^--N fractions obtained from the cold KCl extraction. The hot KCl extraction method was used to measure potentially mineralizable nitrogen (PMN) [48]. In this method, 20 mL of 2 M KCl was added to 3 g of soil, heated to 100 °C for 4 h in a hot water bath, cooled to room temperature, and filtered through a Whatman #42 filter paper. After that, the supernatant was tested for NH_4^+-N by colorimetry. PMN was calculated by subtracting the cold KCl-extracted NH_4^+-N from the hot KCl-extracted NH_4^+-N. Loss-on-ignition carbon (LOI) was measured gravimetrically as the lost weight caused by heating 1 g of soil for 8 h at 550 °C in a Thermolyne muffle furnace (model F6010, Thermo Fisher Scientific Inc., Asheville, NC, USA). Manure samples were digested with H_2SO_4 and analyzed for total Kjeldahl N (TKN) with a colorimeter technique using a TECAN spectrophotometer at 660 nm [49].

2.7. Statistical Analysis

Partition predictive modeling and multivariate correlation tools from JMP were used to establish correlations between the variables and to find possible cut points in the ranges. Regressions for the nitrogen and carbon emissions and soil nitrogen relationships with filth fly numbers, ant activity, and predator numbers were made using simple linear regression ($p < 0.05$). Comparisons between multiple means were determined by nonparametric comparisons for each pair using the Wilcoxon method ($p < 0.05$). All statistical analyses were performed using the JMP software package (JMP®, Version 16. SAS Institute Inc., Cary, NC, USA, 1989–2023).

3. Results and Discussion

3.1. Characterization of Epigeal Arthropods and Filth Fly Populations

Filth fly and epigeal arthropods populations showed a Shapiro–Wilk value lower than 0.05 and were catalogued as not normally distributed. The composition of the captured and emerging filth fly populations during the two-year sampling period is shown in Table 2. The horn fly was the main species laying eggs and emerging from the sampled cattle manure. Hollowed Hawk Farm in Watkinsville showed the highest values of filth fly population, driven mainly by its high values of face flies when compared to the other farms.

Table 2. Composition of the filth fly population captured during the 2021–2022 sampling period. Median and quartile deviation (QD) are shown for every farm.

Species	Eatonton Research Farm		Garmon Farm		Hollowed Hawk Farm		Total Number of Individuals per Species	Percentage of Total
	Total	Median ± QD	Total	Median ± QD	Total	Median ± QD		
Horn fly (*Haematobia irritans* Linnaeus)	384	21.0 ± 12.8	215	10.0 ± 7.9	380	11.5 ± 9.6	979	80.2%
Bottle fly (*Lucilia sericata* Meigen)	32	1.0 ± 1.5	6	0.0 ± 0.4	48	1.0 ± 1.5	115	9.4%
Face fly (*Musca autumnalis* DeGeer)	3.0	0.0 ± 0.0	3	0.0 ± 0.0	109	0.0 ± 1.0	86	7.0%
Eye gnat (*Liohippelates* spp.)	5	0.0 ± 0.0	0	0.0 ± 0.0	35	0.0 ± 0.0	40	3.3%
Total filth flies per farm	424	25 ± 11.0	224	10.0 ± 8.0	572	23.5 ± 10.8	1220	

The composition of predators is given in Table 3. Red imported fire ants were the main species of this functional group, and the Hallowed Hawk Farm showed the highest numbers of total individuals.

Table 3. Composition of the predator population captured during the 2021–2022 sampling period. Median and quartile deviation (QD) are shown for every farm.

Species	Eatonton Research Farm		Garmon Farm		Hollowed Hawk Farm		Total Number of Individuals per Species	Percentage of Total
	Total	Median ± QD	Total	Median ± QD	Total	Median ± QD		
Ants (*Solenopsis invicta* Buren) *	92	9.5 ± 10.0	112	21.0 ± 29.5	184	1.5 ± 12.3	388.0	68.9%
Spiders (*Agelenopsis* spp.)	51	3.0 ± 2.5	10	0.0 ± 0.5	18	0.0 ± 0.5	79.0	14.0%
Tiger beetles (Cicindelinae subfamily)	21	1.0 ± 1.0	24	0.0 ± 0.8	19	0.0 ± 0.5	64.0	11.4%
Paper wasp (Polistinae subfamily)	5	0.0 ± 0.0	6	0.0 ± 0.4	7	0.0 ± 0.0	18.0	3.2%
Rove beetles (Staphylinidae family)	5	0.0 ± 0.0	4	0.0 ± 0.0	5	0.0 ± 0.0	14.0	2.5%
Total epigeal arthropods per farm	174	5.0 ± 6.3	156	4.0 ± 3.4	233	2.5 ± 2.6	563.0	

* Pictures taken during the hot dog test were analyzed by two ant experts from the Entomology Department of the University of Georgia and they characterized the ants as "red imported fire ants" (*Solenopsis invicta* Buren). Ants were considered as the number of ants per square centimeter of hot dog.

3.2. Factors Affecting Filth Fly Development

The number of filth flies emerging from cattle manure had a direct correlation ($p < 0.01$) with the initial N content of the manure only when the number of ants per square centimeter was lower than eight (Figure 3). Filth fly larvae eat manure nutrients and bacteria. Manure with a greater N content has been shown to produce higher-quality larvae and lower larval mortality [50]. During sample collection, ants were constantly seen carrying larvae, pupae, and emerging filth flies from cattle manure. In addition, previous studies have recognized red imported fire ants as filth fly predators [30,51]. We also found that the number of filth flies emerging from cattle manure decreased significantly ($p < 0.01$) when the number of predators per chamber was equal to or greater than five (Figure 4).

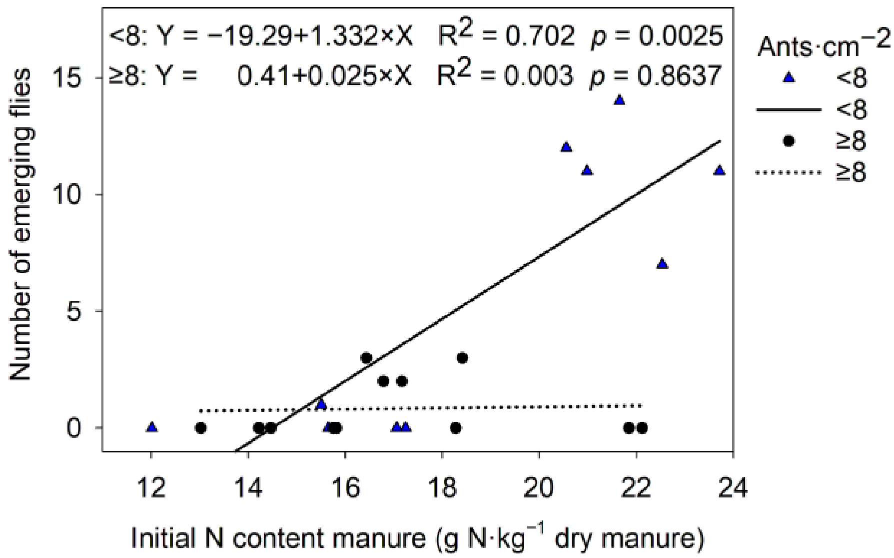

Figure 3. The number of flies emerging from manure (day 15/16) vs. initial nitrogen (day 0) content in manure under two ranges of ant count per square centimeter.

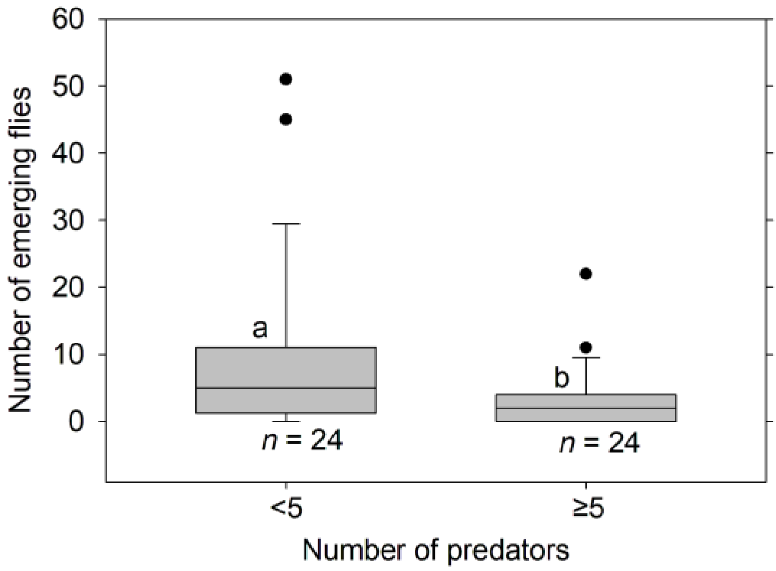

Figure 4. The number of flies emerging from manure per 16-day cycle when predators per chamber were <5 or ≥5. Different letters indicate a significant difference ($p < 0.05$).

3.3. Nitrogen Emissions

3.3.1. Ammonia Emissions

Ammonia emitted during the 16-day sampling (cumulative NH_3) was between 4.1 and 126.4 mg NH_3-N·kg^{-1} manure and represented between 0.01 and 0.7% of the initial N in manure. Bussink also found that there was high variability when measuring NH_3 emissions in rotational dairy systems that received nitrogen fertilizer. They found an average value of 3.1 ± 13% of the initial N in manure was emitted as NH_3 [52]. Nichols et al. found similar results with a high variability depending on rain and grass type [53].

Cumulative NH_3 had a positive relationship ($p < 0.05$) with the total number of flies that laid eggs and emerged from the cattle manure when the average daily temperature was lower than 25 °C. When the average daily temperature was between 23 and 25 °C, every additional fly ovipositing in manure represented an increase of 0.46 mg N-NH_3·kg^{-1} manure emissions. When the average daily temperature was equal to or greater than 25 °C, there was no clear relationship between cumulative NH_3 and the total number of flies, but there was a direct relationship between cumulative NH_3 and the number of ants per square centimeter ($p < 0.05$), in which every additional ant represented an increase of 0.54 mg N·kg^{-1} manure in emissions (Figure 5B). An increase in manure N emissions can be the result of an increase in the air-filled pore space which promotes gas exchange [31,37]. Ant and fly larvae can cause an increase in pore space by their movement, which can create holes or pores in the manure. The increase in emerging filth flies could indicate a greater number of larvae, which would create more pore space and higher NH_3 emissions as seen in Figure 6. Ant activity was lower when the average daily temperatures were lower than 25 °C. Therefore, we speculate that the ammonia emissions when temperatures ranged from 23 to 25 °C were driven mainly by filth fly activity, while at higher temperatures, the ammonia emissions were driven by ant activity. Previous research suggests that the optimum temperature for red imported fire ants to forage is between 25 and 35 °C [54,55].

NH_3 emissions measured on sampling days 0 and 4 were significantly higher than those collected during days 8 and 15 (Figure 6). Horn fly and face fly eggs hatch within a few days of oviposition and larvae feed for the next 2 to 4 days [19,56]. While larvae were not counted, their lifecycle would suggest that larval activity coincides with the greater level of ammonia emissions.

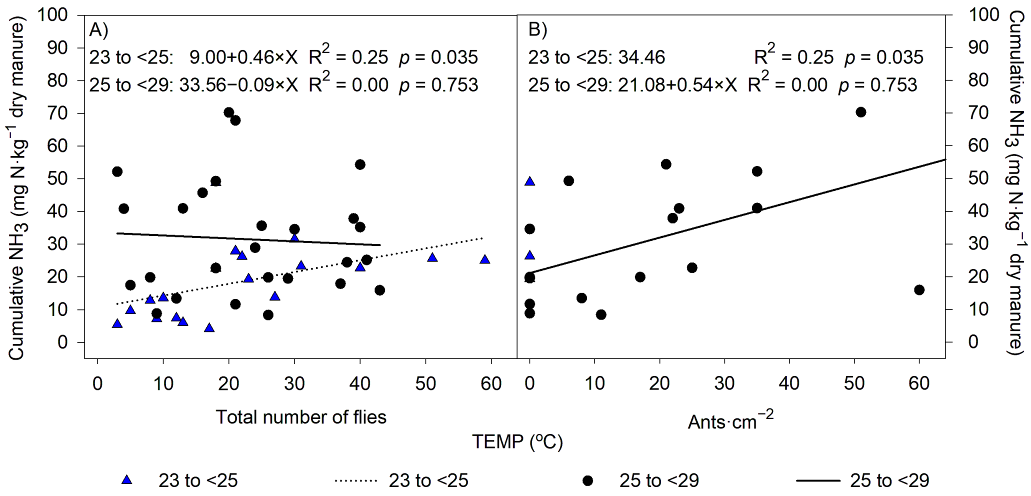

Figure 5. (**A**) Cumulative NH_3 emitted during the 16-day sampling period vs. the total number of flies that oviposited or emerged from manure pat, under two ranges of daily average temperature (23 to <25 °C and ≥25 to <29 °C). (**B**) Cumulative NH_3 emitted during the 16-day sampling period vs. the total number of ants per square centimeter that were recorded per manure pat, under two ranges of daily average temperature (23 to <25 °C and ≥25 to <29 °C).

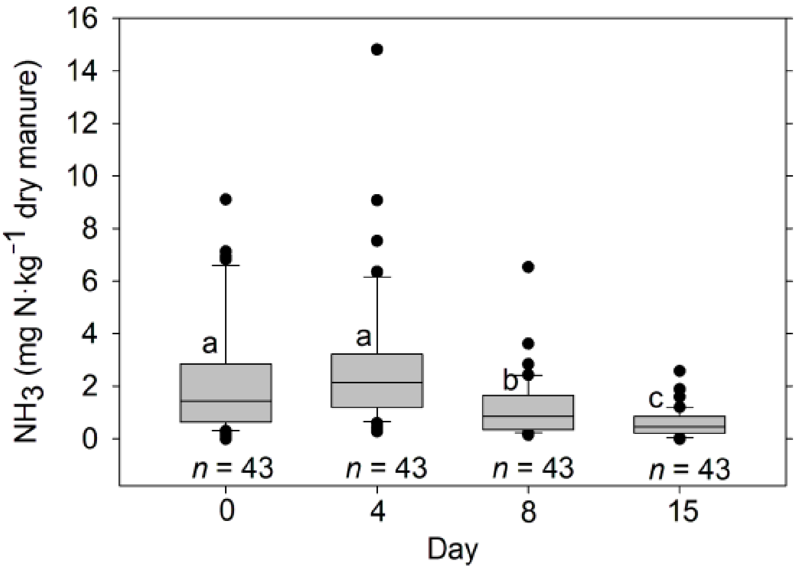

Figure 6. NH_3 emissions measured 0, 4, 8, and 15 days after manure deposition. Different letters indicate a significant difference ($p < 0.05$).

3.3.2. Nitrous Oxide Emissions

Nitrous oxide emitted during the 16-day sampling period (cumulative N_2O) was between 1.4 and 31.5 mg N_2O-N·kg^{-1} manure and represented between 0.1 and 0.6% of the initial N in manure. In a simulated grazing system with no previous fertilization, Yamulki et al. found that N_2O emitted during 100 days after pat deposition was between 0.04 and 0.53% of the initial N in manure [57]. Another study found ranges between 0.05% and 0.07% 78 days after deposition [53].

Cumulative N_2O had different relationships with the number of total flies captured depending on the amount of precipitation received the day before and the day of sampling and whether the manure pat was under tree cover or not. In open pastures, cumulative N_2O had a direct correlation with the total number of flies captured. However, under tree cover, the N_2O emissions increased with fly numbers only when the precipitation level was lower than 31 mm, otherwise the N_2O decreased with the number of flies (Figure 7).

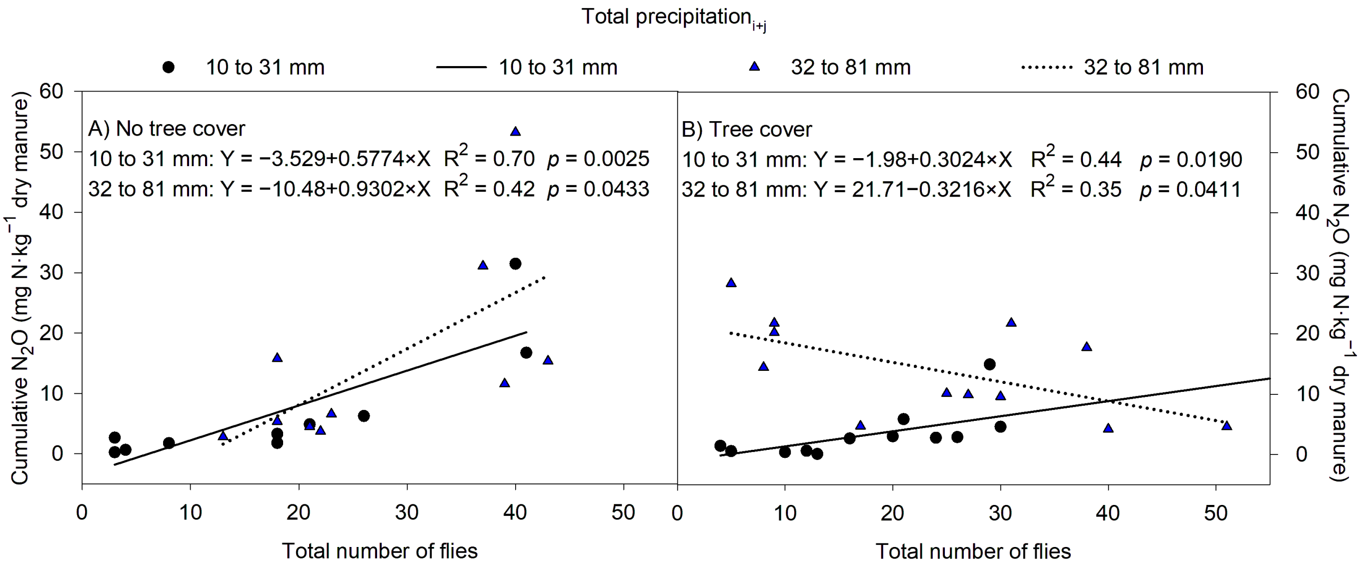

Figure 7. Cumulative N_2O emitted per manure pat during the 16-day sampling period vs. the total number of flies that oviposited or emerged from manure pat, under two ranges of total precipitation$_{i+j}$ (10 to 31 mm and 32 to 81 mm). (**A**) Manure pats were on open pastures without cover. (**B**) Manure pats were under tree cover.

Nitrous oxide is produced under aerobic conditions during the nitrification process, and under anaerobic conditions during the denitrification process [8]. Factors such as high moisture, neutral pH, high temperature, and the presence of soluble organic matter and nitrate promote denitrification [8,16,58]. Most of the N_2O emitted from cattle manure is produced by the denitrification process in the top 0–5 cm layer [59]. The larval burrowing activity can facilitate anaerobic conditions by allowing precipitation water to go into deeper layers of manure and soil, thereby increasing N_2O emissions when it rains. Figure 8 illustrates that cumulative N_2O was significantly higher (p = 0.0001) when total precipitation$_{i+j}$ (Equation (1)) ranged between 32 and 81 mm. Manure pats in open pastures captured more rainfall and, coupled with the greater porosity created from larval and emerging fly activity, pockets of saturation and partial saturation were present, allowing for denitrification to occur.

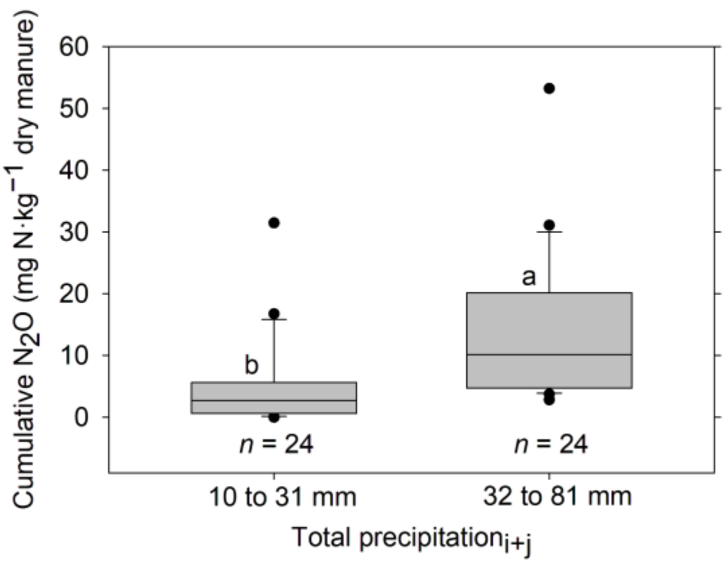

Figure 8. Cumulative N_2O emitted per manure pat during the 16-day sampling period under two ranges of total precipitation$_{i+j}$ (10 to 31 and 31 to 81 mm). Different letters indicate a significant difference (p < 0.05).

Manure pats under tree cover likely received less precipitation because some of the rain was intercepted by leaves and limbs and fell to the ground as throughfall, or when smaller rainfall events occurred, most water was retained by leaves and branches. As such, on manure pats under tree cover and receiving less precipitation, the larval activity could promote N_2O emissions by the nitrification process, by increasing gas exchange and oxygen availability in deeper layers of manure, similar to the effect on ammonia emissions. The water-filled pore space (WFPS) and oxygen content are key factors in determining the nature of the N_2O emission process in manure, changing from a denitrification to nitrification process when oxygen concentration is more than 12% [37,60,61]. In heavier rainfall events under trees, N_2O emissions had a negative relationship with the total number of flies; we speculate that most of the rainfall was throughfall, and manure pats did not receive the amount of rain needed for pats to become saturated and induce denitrification. There was not enough water to saturate the created holes to facilitate denitrification and there was not enough oxygen to promote nitrification. Ammonium oxidation is the first step in the nitrification process and actively consumes oxygen. Under laboratory conditions, increasing the numbers of fly larvae has been shown to decrease N_2O emissions by consuming nitrogen sources and increasing carbon levels [22].

3.3.3. The Impact of Filth Flies on Nitrogen Emissions in the Environment

To determine the impact of the presence of filth flies on N emissions in a wider region, we computed the potential effect on monthly N emissions for both a farm and the state of Georgia. We considered that for every extra fly on open pastures, the NH_3 emissions increase in 0.46 mg $N \cdot$ kg dry manure^{-1} (Figure 5A), and N_2O emissions increase between 0.58 and 0.93 mg $N \cdot$ kg dry manure^{-1} depending on the amount of rain received (Figure 7A). The median and quartile deviation for the total number of flies per manure pat in our study was 20 ± 11, the average manure moisture was 87%, and the cattle density was one cow per ha. In addition, every beef cow produces an average of 56.8 kg of fresh manure per day [62]. The state of Georgia has 11,025 small family farms (with a gross cash farm income below $150,000) that produce beef cattle in an average area of 51.4 ha each [12].

The quantity of emissions caused only by filth flies coming from small farms in Georgia can vary depending on the weather conditions and the fly infestation. In drier conditions, it can reach up to 27.3 tons per month, while during rainy conditions, it can go up to 36.5 tons per month (Table 4). These are additional emissions to the conventional manure emissions.

Table 4. N emissions produced by filth fly presence at a small-farm scale and on a state scale.

Rain Range (mm)	N Produced by Filth Flies (mg N $\cdot$ Day^{-1} Fly^{-1} $\cdot$ kg Dry Manure^{-1}) *	N Emitted per Cow (g N $\cdot$ Day^{-1})		N Emitted per Farm (kg N $\cdot$ Month^{-1})		N Emitted in Georgia (ton N $\cdot$ Month^{-1})	
		When 9 Flies per Manure Pat	When 31 Flies per Manure Pat	When 9 Flies per Manure Pat	When 31 Flies per Manure Pat	When 9 Flies per Manure Pat	When 31 Flies per Manure Pat
10 to 31	1.04	0.5	1.6	0.7	2.5	7.9	27.3
32 to 81	1.39	0.6	2.1	1.0	3.3	10.6	36.5

* This accounts for NH_3 and N_2O emissions.

3.4. Carbon Dioxide Emissions

The cumulative CO_2 emitted during the 16-day sampling period (cumulative CO_2) was between 0.1 and 136.5 g $CO_2 \cdot kg^{-1}$ manure. Hanafiah et al. found cumulative CO_2 emissions between 30.5 and 50.9 g $CO_2 \cdot kg^{-1}$ manure after 8 days of sampling under laboratory conditions (units were transformed from percentages into g per kg of dry manure considering manure moisture of 85%) [63].

The cumulative CO_2 emitted during the 16-day sampling period had a direct relationship with the number of ovipositing flies only when the average temperature was <25 °C and the range of total precipitation$_{i+j}$ (Equation (1)) was lower than 31 mm (Figure 9A). When the average temperature was greater than 25 °C the ants were more active and had a direct relationship with the cumulative CO_2 emitted during the 16-day sampling period, but only when the range of total precipitation$_{i+j}$ was lower than 31 mm (Figure 9B). Carbon dioxide emissions under the higher precipitation range (32 to 81 mm) did not show any relationship with the number of ovipositing flies or ants per square cm. Similar to NH_3, an increase in CO_2 production with filth fly and ant numbers might be caused by their burrowing activity since the holes bring additional aeration to the manure. More oxygen and higher temperatures increase aerobic microbial activity and gas diffusion processes in cattle manure [33,64]. Fresh manure has high moisture levels (87% average) that cause a low gas diffusion and create a reduced oxygen supply under the manure surface. If media porosity increases and the precipitation range is lower than the volume of air-filled pore space, then the overall oxygen supply increases [37,65]. This may explain why the relationships between CO_2 emission and number of ovipositing flies existed only when the precipitation ranges were lower than 31 mm. Other researchers have also found a positive correlation between insect activity on cattle manure and CO_2 emissions [22,66], and between higher soil temperatures and CO_2 emissions during the summer season [9].

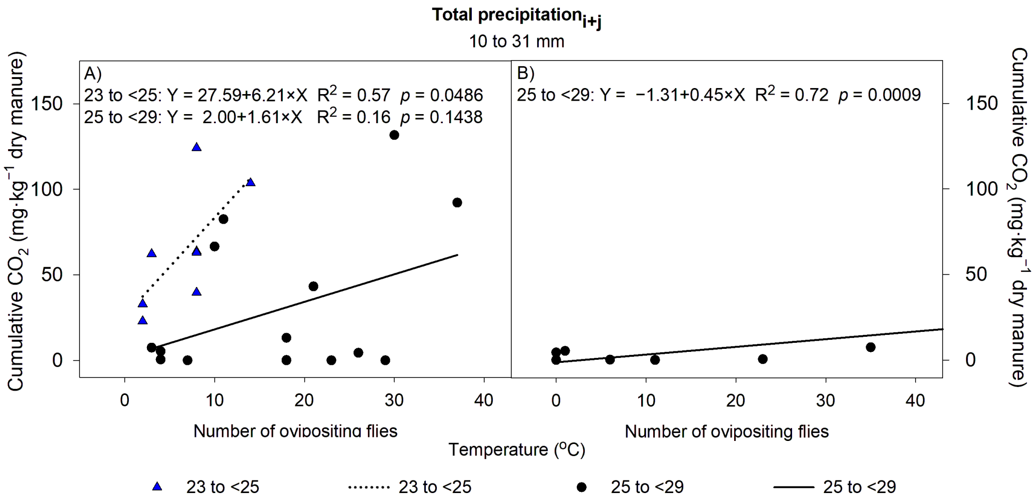

Figure 9. (**A**) Cumulative CO_2 emitted per manure pat during the 16-day sampling period vs. the total number of ovipositing flies per manure pat, under a total precipitation$_{i+j}$ range from 10 to 31 mm, and two ranges of daily average temperature (23 to <25 °C and ≥25 to 29 °C). (**B**) Cumulative CO_2 emitted per manure pat during the 16-day sampling period vs. the total number of ants per square centimeter that were recorded per manure pat, under a total precipitation$_{i+j}$ range from 10 to 31 mm, and two ranges of daily average temperature (23 to <25 °C and ≥25 to 29 °C).

Carbon dioxide emissions also had a direct impact (p < 0.0001) on the loss in manure TKN nitrogen content (initial manure nitrogen content − final manure nitrogen content) (Figure 10). CO_2 emissions are produced by microbial activity consuming cattle manure nitrogen [67]. As microbial activity increases, a greater amount of nitrogen (N) is consumed from manure, resulting in a reduction of organic N. This microbial activity also generates mineralization, which further decreases organic N, while a portion of it is lost through emissions of NH_3 and N_2O.

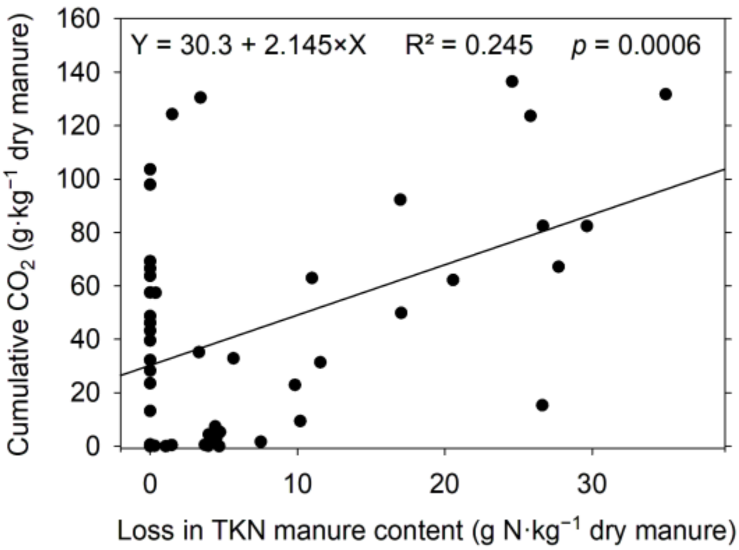

Figure 10. Cumulative CO_2 emitted per manure pat during the 16-day sampling period vs. loss in manure TKN content.

3.5. Soil Nitrogen

Soil nitrate (NO_3^-) measured at the 0 to 5 cm depth (Initial NO_3^- 0–5 cm) on the deposition day was between 11.5 and 276.2 mg N·kg^{-1} soil, and for day 16, it was between 17.75 and 278.85 mg N·kg^{-1} soil. Soil NO_3^- (0–5 cm) did not show a significant difference in open pastures or under tree cover on any of the sampling days. Soil nitrate measured on day 16 (Final NO_3^- 0–5 cm) showed a direct relationship with arthropod diversity measured by the Shannon index but only on pastures that received 32 to 81 mm of precipitation during the 16-day sampling period (Figure 11). No correlation was found between initial soil NO_3^- and arthropod diversity according to the Shannon index. No significant differences were indicated in initial soil NO_3^- between the two precipitation ranges. The Shannon index shows the diversity of species in the community; in this case, it showed the diversity between the six categories of arthropod functional groups. The deposition of insect frass and cadavers can influence nutrient cycling in soils, and their fecal material can have higher nitrogen concentration than the original content they ate [26,68]. Nutrients such as NO_3^- can travel with rain; Tukey and Morgan found that insect damage to plants could change the nutrient content of precipitation falling through damaged plants [69]. Swank et al. found a relationship between insect defoliation and an increase in stream nitrate after winter [70]. In addition, plant-available nitrogen concentration is higher in ant nests, and dung beetle activity can accelerate the mineralization of organic nitrogen found in cattle manure [27,28]. Since no significant difference was shown for any individual functional category, it might be possible that the increase in NO_3^- at the 16-day point was the result of the interactions between the functional categories, helped by the carrying capacity of rain.

Soil ammonium (NH_4^+) measured for the 0 to 5 cm depth was between 0.1 and 494.8 mg NH_4^+-N·kg^{-1} soil at day 0 and between 0.8 and 174.8 mg NH_4^+-N·kg^{-1} soil at day 16. No significant difference was found in the initial NH_4^+ between samples taken from open pastures and under tree cover (Figure 12A). However, day 16 NH_4^+ (0–5 cm soil depth) content was significantly higher ($p < 0.03$) when the number of predators captured in the surrounding pitfall traps was $\geq$5 (Figure 12B). LaFleur et al. found that the soil from nests of red imported fire ants had higher concentrations of NH_4^+, and Berg et al. showed the important contribution of spiders to N mineralization [27,71]. No significant difference was found on NH_4^+ at two ranges of fire ant numbers, spiders, herbivores, filth flies, or any other functional group by itself. Osler and Sommerkorn described that in ecosystems with N limitations, the microbes tend to immobilize any source of N that they receive, while soil fauna contributes to the release of NH_4^+ by grazing on the microbial biomass [72]. Grazing systems with no fertilization count as ecosystems with N limitations; while microbes can immobilize N as biomass, they are also the foundation of the food web that in this ecosystem includes as a minimum dung beetles and filth fly larvae eating manure and microbial biomass, which are then consumed by predators such as fire ants and tiger beetles. In addition, the activity of predation causes a manure disturbance, resulting in a dual biotic influence on N mineralization and soil N concentration. This dual impact might be the most probable explanation for the increase in soil NH_4^+.

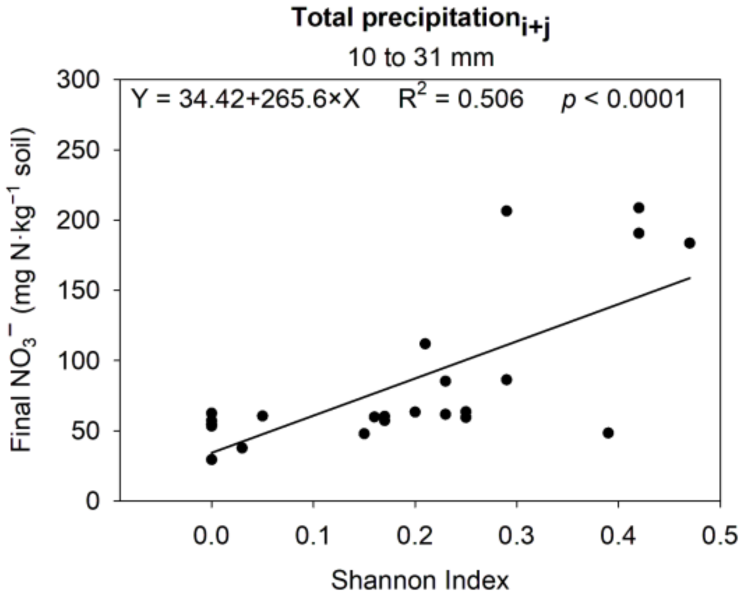

Figure 11. Final soil NO_3^- (0–5 cm depth 16-day) vs. Shannon index value under a total precipitation$_{i+j}$ range from 32 to 81 mm.

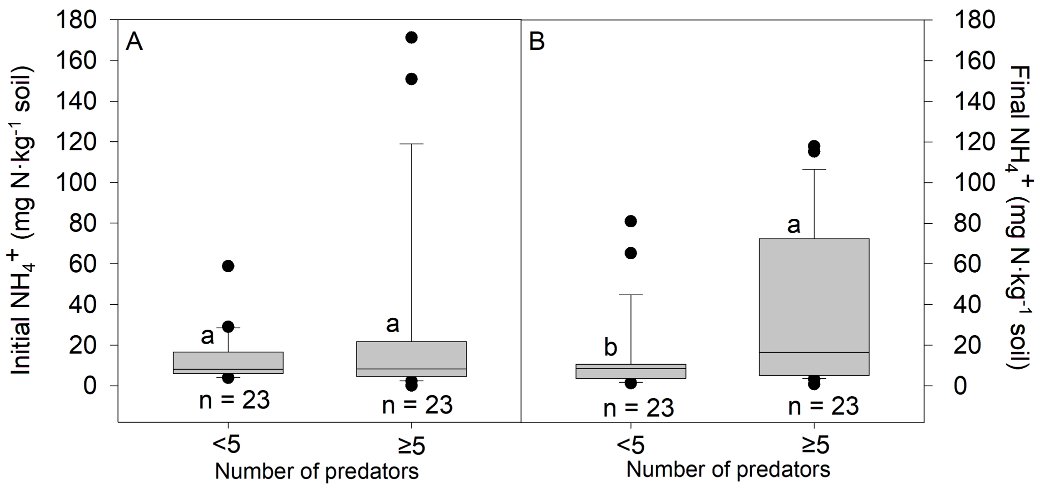

Figure 12. (**A**) Initial soil NH_4+ and (**B**) final soil NH_4+ (0–5 cm depth) when predators per chamber were <5 or ≥5. Different lowercase letters indicate a significant difference ($p < 0.05$).

Plant-available nitrogen (PAN) for the 0 to 5 cm soil depth did not show a significant difference between the samples taken on open pastures and under tree cover. The ranges of results were 16.2–293.1 and 34.9–395.8 mg N·kg^{-1} soil for the samples taken on days 0 and 16, respectively. As calculated, the change in plant-available nitrogen (ΔPAN = PAN at 16 days − PAN at 0 days) is positive when the soil has gained plant-available nitrogen.

In the 0 to 5 cm soil depth, ΔPAN was significantly lower ($p < 0.03$) and negative when the number of filth flies ovipositing on the surrounding manure pat was equal to or more than 14, but only when the manure pat was on open pastures (Figure 13A). When the manure pat was under tree canopy, the number of filth flies ovipositing on it did not affect the ΔPAN 0–5 cm (Figure 13B). A greater number of ovipositing flies meant more larval activity and higher N_2O emissions from manure pats on open pastures, but it had a mixed effect on manure pats under tree cover (Figure 8).

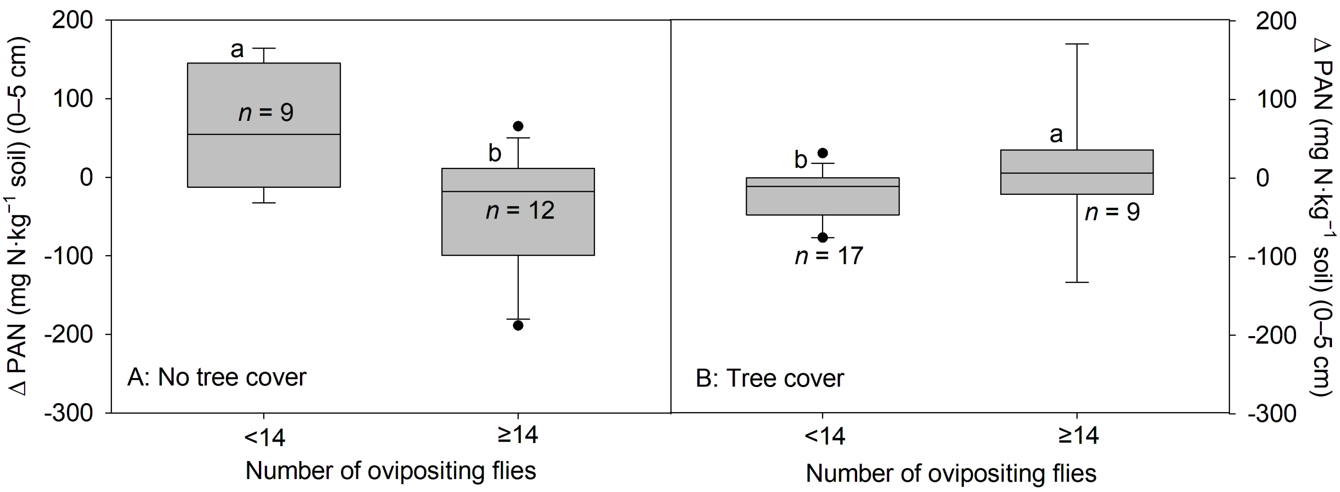

Figure 13. Soil ΔPAN (change in plant-available N, 0–5 cm depth) when ovipositing flies per chamber were <14 or ≥14. (**A**) Manure pats that were under no cover, and (**B**) manure pats that were under tree cover. Negative numbers indicate a loss in PAN and different lowercase letters indicate a significant difference (p < 0.05).

Potentially mineralizable nitrogen (PMN) from the 0 to 5 cm soil depth did not show a significant difference between the samples taken on open pastures and under tree cover. The ranges of results were 1.9–152.3 and 5.1–150.6 mg N·kg^{-1} soil for the samples taken on days 0 and 16, respectively. The change in potentially mineralizable nitrogen (ΔPMN = PMN at 16 days − PMN at 0 days) reflects the soil's gain or loss of easily decomposable nitrogen that microorganisms convert into NH_4^+. A positive ΔPMN value indicates the soil has gained potentially mineralizable nitrogen.

ΔPMN from the 0 to 5 cm soil depth had a negative correlation with the number of filth flies ovipositing on the manure pats that were under no cover (Figure 14). The number of flies ovipositing did not affect the ΔPMN 0–5 cm on manure pats that were under tree canopy. From the linear regression, it is apparent that a number of filth flies on manure greater than 12 produced a loss of PMN. Considering that our median and quartile deviation was 11 ± 8 ovipositing flies per manure pat, when 19 filth flies ovipositing per manure pat were considered, they could cause a loss of up to 14.7 kg N per hectare in PMN. A greater number of ovipositing flies meant a greater larval activity, consuming N from manure and causing higher N emissions (in the case of the manure that was on open pastures). Macqueen and Beirne found a relationship between increasing numbers of filth fly larvae and the loss of nitrogen in cattle manure [73].

Measures of PMN on days 0 (initial PMN 0–5 cm) and 16 (final PMN 0–5 cm) were significantly higher (p < 0.03) when the number of predators captured over the 16-day period for each chamber (pitfall trap or hotdog) was equal to or greater than five (Figure 15). It has been discussed how predators can increase NH_4^+ in soil. However, the fact that the initial and final PMN contents were higher when greater numbers of predators were present shows the link between the initial increase in PMN and how it ends as higher NH_4^+ concentrations. After all, NH_4^+ is linked to the decomposition of organic matter, and arthropod communities can explain between 11 and 15% of the variation in the decomposition of organic matter [74].

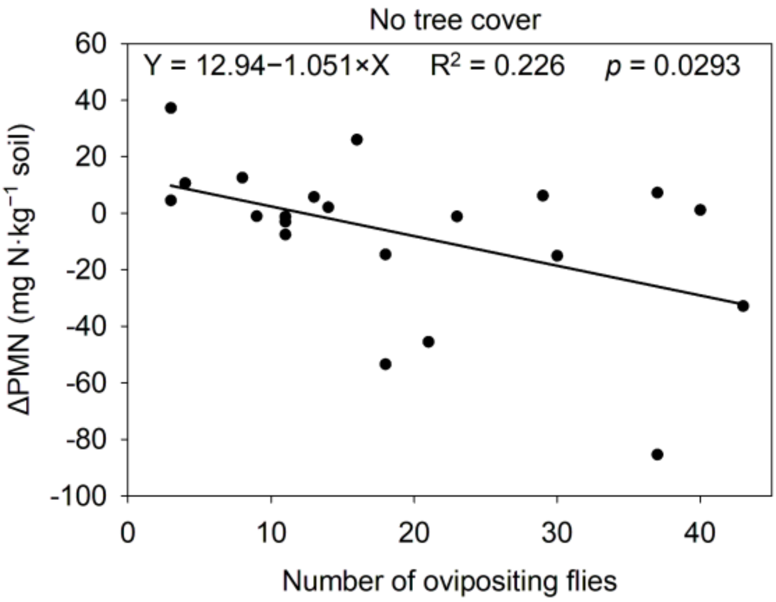

Figure 14. Soil ΔPMN (change in potential mineralizable N, 0–5 cm depth) vs. number of ovipositing flies on manure pats that were under no cover.

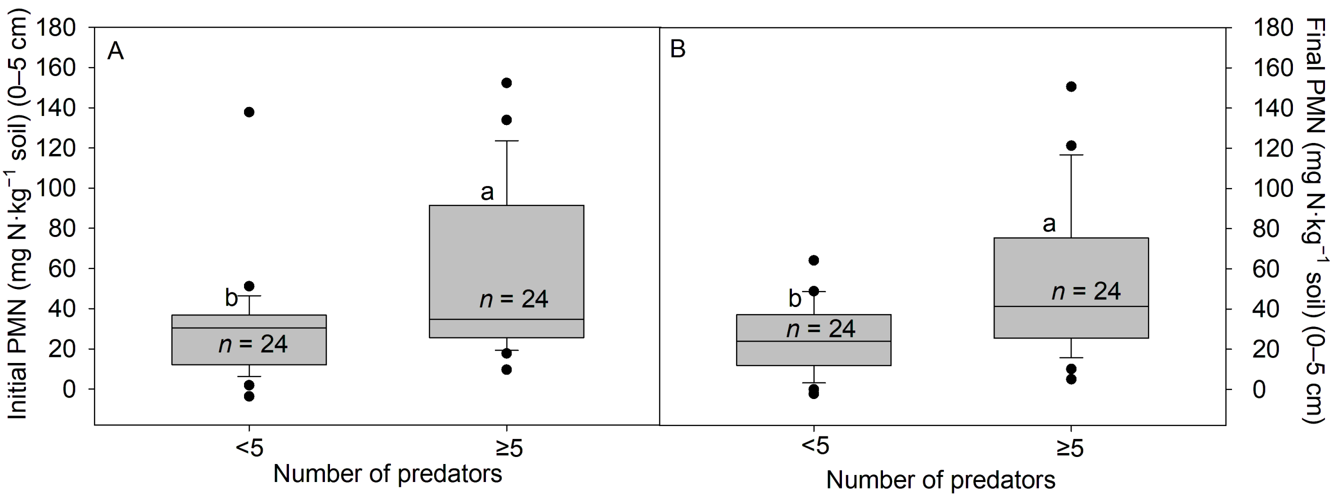

Figure 15. (**A**) Initial soil PMN and (**B**) final soil PMN (0–5 cm depth) when predators per chamber were <5 or ≥5. Different lowercase letters indicate a significant difference ($p < 0.05$).

4. Conclusions

The horn fly was the main species laying eggs and emerging from the sampled cattle manure, while the red imported fire ants showed the highest numbers under the predator category. The number of filth flies emerging from cattle manure had a direct correlation with the initial nitrogen content in the manure only when the number of ants per square centimeter was lower than eight. An increasing number of flies ovipositing and emerging from cattle manure, as well as red imported fire ant activity, has the capability of increasing NH_3, N_2O, and CO_2 emissions. Considering only from the small cattle farms in Georgia, filth flies could be depleting those farms of 36.5 ton of N as N emissions to the atmosphere. The relationships between cumulative NH_3, cumulative N_2O, and cumulative CO_2 and the number of flies and ants were affected by temperature and precipitation levels. The final soil NO_3^- and NH_4^+ levels were affected by precipitation levels and the number of predators. ΔPAN and ΔPMN in the soil were negatively correlated with the number of flies ovipositing on the manure on open pastures, while initial and final soil PMN contents were positively correlated with the number of predators found in the area. Ovipositing filth flies could cause a loss of up to 14.7 kg N per hectare in the PMN; this could be a crucial factor for small-scale farming systems that do not receive any fertilization. From this, we may conclude that the filth fly presence in grazing systems with no fertilization legacy can negatively affect soil nitrogen retention, while an increase in the biodiversity and abundance of predators may help to increase PAN and PMN in open pastures, as well as decreasing the numbers of emerging filth flies.

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