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Influence of Red Imported Fire Ants on Greenhouse Gas Emissions from a Piedmont Plateau Pasture

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ABSTRACT

Researchers have previously attempted to relate the influences of many ant species to the nitrogen (N) and carbon (C) cycles. However, the gaseous phases of these cycles have been neglected. A field study was conducted near Auburn University $(32^{\circ}52'N, 85^{\circ}30'W)$ in a bermudagrass [*Cynedon dactylon* (L.) Pers] pasture to determine the influence of the red imported fire ant [*Solenopsis invicta* (L.) Buren] on soil emission of three greenhouse gases from April 1999 to April 2000. A completely random design was used with three replications. Treatments consisted of ant influenced soil (mounds) and non-ant influenced control soil. Surface emission rates of N₂O-N, CH₄-C, and CO₂-C were measured bi-weekly using a closed chamber technique. Soil collection to a depth of 100 cm

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was used to determine soil moisture, pH, and status of soil N and C. While the red imported fire ant significantly influenced greenhouse gas fluxes from mound soil, it was concluded that for a bermudagrass pasture in Alabama, the increase of annual emissions of N₂O-N, CH₄-C, and CO₂-C was only 6.95 g N ha^{-1} , 0.16 g C ha^{-1} , $0.92 \text{ kg C ha}^{-1}$, respectively. It is speculated that regional and global budgets of these greenhouse gases may be underestimated when the influence of soil macro-organisms, such as ants, are not taken into account. However, this underestimation may only be slight.

Key Words: Red imported fire ants; Nitrous oxide emission; Carbon dioxide emission; Methane emission; Soil carbon; Soil nitrogen.

INTRODUCTION

There are presently 9,500 identified species of ants around the world, and it is estimated that nearly 20,000 more species have yet to be identified.^[1] It has also been estimated that, with an extensive range from tropic to temperate zones, the mass of the nearly ten trillion ants on earth equals that of all humans.^[1] The force of these insects greatly influences the general area in which their colonies are found, and there currently exists a large body of research describing these localized effects. However, the impact of these organisms on the global environment through contributions to the greenhouse effect has not yet been addressed.

During an intact core field study of greenhouse gas emissions from swine waste amended soils, evidence seemed to indicate that the red imported fire ant, *Solenopsis invicta*, could increase N₂O-N emissions considerably from a Norfolk loamy sand (fine, loamy, siliceous, thermic, Typic Kandiudult) (unpublished data). It was discovered that the mean N₂O-N emission rate from one control core disturbed by an established fire ant colony was $232 \,\mu g \, N_2 O$ -N m⁻² h⁻¹, as compared to a mean rate of $12 \,\mu g \, N_2 O$ -N m⁻² h⁻¹ from all undisturbed control cores. There is currently a lack of research to substantiate these accidental findings, however, a large body of research addressing the effects of ants on soil N is available.

Ant colony soil typically has higher rates of N mineralization, and contains greater concentrations of NO₃-N and NH₄-N than soil not influenced by ants.^[2-4] These foraging insects are capable of significantly redistributing N in their environment. In a study of plant–ant mutualism, Treseder et al.^[5] reported that a species of epiphyte (*Dichidia major*) derived 29% of its N from organic material deposited by ants of

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the genus *Philidris*. Similarly, Soyunov^[2] reported that the mounds of three ant species (*Cataglyphis setipes*, *Messor varabilis*, and *M. aralocaspius*) in Russia contained nearly double the amount of N as surrounding soil.

Increased N mineralization rates found in ant mounds may be explained 87 by the gathering and mixing of large quantities of organic material in colonies 88 by ants. Mixing stimulates microbial activity in soil, which in turn, increases 89 degradation of organic material brought into the mounds.^[6–8] Higher rates of 90 mineralization and nitrification could possibly lead to an increase of N2O-N 91 production via denitrification. The rate of denitrification follows Michaelis-92 Menten kinetics, therefore N₂O-N fluxes increase with increasing concen-93 trations of soil NO₃-N to a point in which emission becomes constant 94 regardless of NO₃-N concentration.^[9] However, denitrification rates are also 95 influenced by other factors, such as soil structure and moisture content, which 96 is also be affected by ants.^[10] 97

As with the relationship between ants and N₂O-N emission, there is no 98 documentation of the influence of ants on either the emission or oxidation of 99 CH₄-C. However, there exists a great deal of research describing 100 the production of CH₄-C by many species of termites.^[11,12] Termite released 101 CH₄-C, which is produced by symbiotic microorganisms found in the hindgut, 102 contributes approximately 5% of the annual global flux of CH_4 -C.^[12] If it is 103 found that CH₄-C is emitted from fire ant mounds, the emission may be a 104 product of a metabolic process within the ant, or caused by soil microbial 105 activity as influenced by ants. 106

Dauber and Wolters^[13] observed CO₂-C evolution rates 1.7 to 2.7 107 times greater from the mounds of three ant species (Myrmica scabrinodis, 108 Lasius niger, and L. flavus) versus non-ant influenced soil. However, 109 Lenoir et al.^[4] observed that CO₂-C evolution from ant mounds was only 110 significantly greater than the forest floor at higher water holding 111 capacities. At a water holding capacity of 60%, Lenoir et al.^[4] reported 112 that ant mound soil increased CO₂-C evolution as great as 1.5 times that 113 of forest floor soil. 114

Solenopsis invicta is one of the common fire ant species inhabiting the 115 southern United States, and it can often be found in fields, pastures, and 116 occasionally wooded areas. First introduced to Mobile, AL from South 117 America around 1930, red imported fire ant populations have steadily 118 increased, and can currently be found in 11 U.S. states.^[14] The objective of 119 this study was to quantifying suspected greenhouse gas emissions from soil 120 influenced by this species of ant. By doing so, contributions of N₂O-N, CH₄-C, 121 and CO₂-C emissions made by ants around the world might be estimated in an 122 effort to improve global greenhouse gas budgets. 123

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MATERIALS AND METHODS

A 12-month study of greenhouse gas emissions from red imported fire ant mounds was conducted in a bermudagrass pasture near Auburn, Alabama, USA (32°41′N, 85°30′W) on a Hiwassee sandy loam (fine, koaolinitic, thermic Typic Rhodudult). Treatments consisted of ant influenced soil (mounds) and non-ant influenced control soil. The experiment was arranged in a completely randomized design having three replications.^[15]

132 Three ant mounds were chosen at random within the bermudagrass pasture during each bi-weekly collection time from April 1999 to April 133 2000. Control sites were not chosen closer than 5 m to any mound. 134 Measurements of N₂O-N, CH₄-C and CO₂-C from the soil surface were 135 made using a closed chamber method.^[16] Chambers were constructed from 136 PVC pipe (20 cm dia. × 16 cm headspace), and were fitted at the top with 137 a vent (5 mm dia. \times 25 cm length), and a sampling port. Chambers were 138 colored white to minimize temperature variation of air within the 139 chambers. Using a syringe, gas samples were collected from the chamber 140 headspace at 0, 30, and 60-minute intervals, and stored in 3 ml vials. Gas 141 samples were analyzed using a Varian star cx gas chromatograph (Varian, 142 Walnut Creek, CA). Nitrous oxide concentrations were determined using a 143 4 m Haysep R column and a ⁶³Ni electron capture detector (ECD). 144 Detector temperature was 350°C, carrier gas was N₂, and carrier gas flow 145 was 17 mL min⁻¹. Carbon dioxide concentrations were determined using a 146 4-m Haysep R column and a thermal conductivity detector (TCD). 147 Detector temperature was 200°C, carrier gas was N₂, and carrier gas flow 148 was 17 mLmin^{-1} . Methane concentrations were determined using a 3 m 149 Porapak N column and a flame ionizing detector (FID). Detector 150 temperature was 350°C, carrier gas was N2, and carrier gas flow was 151 $30 \,\mathrm{mL}\,\mathrm{min}^{-1}$. 152

Soil samples were collected at 5 cm increments to 20 cm, and at 10 cm 153 increments from 20 to 100 cm depth from ant influenced and control soils. Soil 154 samples were stored at 5°C until analysis. Soil pH, moisture, NH₄-N, NO₃-N, 155 total N, and organic C were measured. Soil moisture was measured 156 gravimetrically. Soil was extracted with 2 M KCl, and NH₄-N, and NO₃-N 157 concentrations were determined using a microplate reader.^[17] Soil organic C 158 and total N were determined with a LECO CHN-600 analyzer (LECO Corp., 159 T1 St. Joseph, MI) (Table 1). 160

161 In an effort to quantify greenhouse gas fluxes from fire ant influenced soil, 162 a survey of mound numbers and sizes was conducted every 3 months. Three 163 plots $(30 \times 30 \text{ m})$ within the pasture were established, and counts of mounds 164 and measurements of mound diameters were made.

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Table 1. Initial and final control soil characteristics from fire ant study site near Auburn, AL from April 1999 to April 2000 (0-20 cm).

			Soil N		Soil C	
	pl Initial	H Final	Initial $(g kg^{-1})$	Final $(g kg^{-1})$	Initial $(g kg^{-1})$	Final (g kg ⁻¹)
Bermudagrass pasture soil	5.92	5.99	0.29	0.34	19.7	19.6

Statistical analyses consisted of analysis of variance and least significant difference. Statistical significance was set at the $\alpha = 0.05$ level.

RESULTS

Nitrous Oxide

During our study, a sampling date × treatment interaction occurred for T2 N₂O-N data (P ≤ 0.0001) (Table 2). Eighteen of the 23 sampling dates showed no significant differences between N₂O-N emissions from ant influenced and control soils (LSD_{0.05} = 46 µg N₂O-N m⁻² h⁻¹). Differences between treatments were only observed between May 19 and July 14, and in F1 each case, ant influenced soil emitted more N₂O-N than control soil (Fig. 1). The mean flux of N₂O-N across all sampling dates was 9 and 149 µg N₂O-N m⁻² h⁻¹ for control and ant influenced soils, respectively. Much of the cumulative emissions observed from ant influenced soil was explained during

Table 2. Analysis of variance for effect of sampling date and treatment on N₂O-N emission ($\mu g N m^{-2} h^{-1}$), CH₄-C emission ($\mu g C m^{-2} h^{-1}$), and CO₂-C emission ($m g C m^{-2} h^{-1}$) for control and ant influenced soils near Auburn, AL from April 1999 to April 2000.

199		P > F			
200 201	Source of variance	N ₂ O-N	CH ₄ -C	CO ₂ -C	
202	Date	0.0001	0.2579	0.0001	
203	Treatment	0.0028	0.0211	0.0001	
204	Date*treatment	0.0001	0.0924	0.0001	
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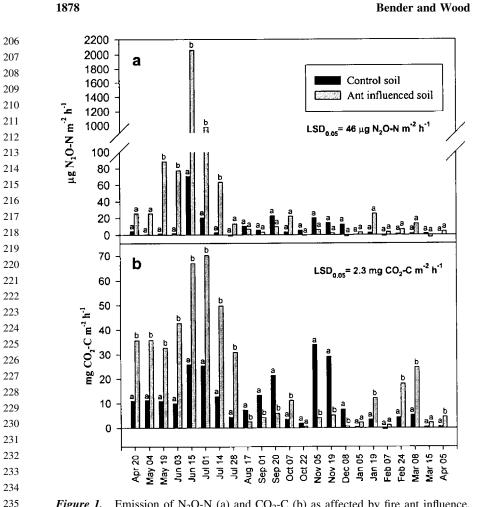


Figure 1. Emission of N₂O-N (a) and CO₂-C (b) as affected by fire ant influence. For each sampling date, values followed by the same letter are not significantly different by LSD_{0.05}. A break occurs on the Y axis of N₂O-N graph between 100 and 1000 μ g N₂O-N m⁻² h⁻¹.

the period between May 19 and July 14. The mean rate of emission from ant influenced soil during this period was $654 \mu g N_2 O \cdot N m^{-2} h^{-1}$ per sampling 242 date. The highest rate of emission $(2060 \,\mu g \, N_2 O \cdot N \, m^{-2} \, h^{-1})$ was observed on 243 June 15. The rate during this day was twice that of the second highest rate of 244 emission found on July 1. Following July 14, mean emission rates of N2O-N 245 from ant influenced soil dropped to $7 \mu g N_2 O-N m^{-2} h^{-1}$. 246

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Table 3. Analysis of variance for effect of sampling date, treatment and soil depth on soil C ($g C kg soil^{-1}$), soil N ($g N kg soil^{-1}$), soil NH₄-N(mg N kg soil⁻¹), and soil NO₃-N (mg N kg soil⁻¹) for control and ant influenced soils near Auburn, AL from April 1999 to April 2000.

	P > F				
Source of variance	Soil C	Soil N	Soil NH ₄ -N	Soil NO ₃ -N	
Date	0.0001	0.0001	0.0001	0.0001	
Treatment	0.0001	0.0946	0.0001	0.0001	
Depth	0.0001	0.0001	0.0202	0.0001	
Date*treatment	0.0029	0.0001	0.0001	0.0001	
Date*depth	0.0001	0.0001	0.9145	0.9892	
Treatment*depth	0.0001	0.0001	0.0271	0.0001	
Date*treatment*depth	0.0001	0.0001	0.7225	0.9995	

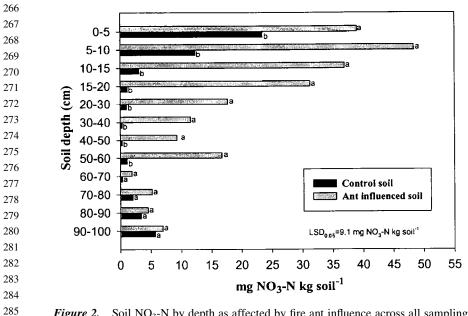


Figure 2. Soil NO₃-N by depth as affected by fire ant influence across all sampling dates. For each soil depth, values followed by the same letter are not significantly different by LSD_{0.05}.

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As with rates of emission from ant influenced soil, the highest N₂O-N emission rates from control soil were also observed during the period between May 19 and July 14. The rates of emission from control soil during this period averaged $20 \,\mu g \, N_2 O$ -N m⁻² h⁻¹, and the rate of emission found on June 15 was nearly 3.5 times greater than the sampling date with the next highest rate of emission (July 1). Following July 14, mean emission rates of N₂O-N from control soil dropped to $6 \,\mu g \, N_2 O$ -N m⁻² h⁻¹; a rate quite comparable to that of ant influenced soil following July 14.

Other factors that influence N₂O-N fluxes, such as soil NO₃-N and NH₄-N, and soil pH, were also documented during this study. Significant sampling depth × treatment interactions were observed for soil NO₃-N (P \leq 0.0001) and **T3** soil NH₄-N (P \leq 0.0271) (Table 3). Ant influenced soil had significantly higher concentrations of soil NO₃-N and NH₄-N for all depths between 0–60 cm **F2** (Figs. 2 and 3). Mean NO₃-N concentrations from 0–60 cm were 4.8 and 23.6 mg NO₃-N kg soil⁻¹ for control and ant influenced soils, respectively. Mean NH₄-N concentrations from 0–60 cm were 3.78 and 18.5 mg NH₄-N kg soil⁻¹ for control and ant influenced soils, respectively. Also observed was a significant

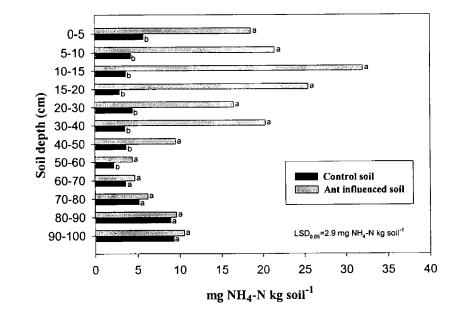


Figure 3. Soil NH₄-N by depth as affected by fire ant influence across all sampling dates. For each soil depth, values followed by the same letter are not significantly different by LSD_{0.05}.

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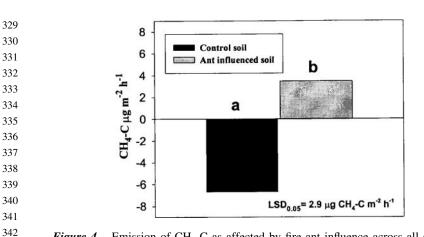
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Figure 4. Emission of CH₄-C as affected by fire ant influence across all sampling dates. Values followed by the same letter are not significantly different by LSD_{0.05}.

treatment effect for soil pH ($P \le 0.013$). The mean soil pH of control soil during this study was 5.9, as compared to 5.6 for ant influenced soil.

Methane

A significant treatment effect (P ≤ 0.0211) occurred for CH₄-C emission data during this study (Table 2). Unlike the N₂O-N data, a definable seasonal pattern of higher rates of CH₄-C emission or oxidation was not observed. The flux of CH₄-C was quite variable throughout the study, with a range of emissions for each treatment over all sampling dates between - 39 to 17 µg CH₄-C m⁻² h⁻¹, and -24 to 39 µg CH₄-C m⁻² h⁻¹ for control and ant influenced soils, respectively. Across all sampling dates, ant influenced soil emitted an average of 3.5 µg CH₄-C m⁻² h⁻¹, while control soils oxidized an average of 6.7 µg CH₄-C m⁻² h⁻¹ (LSD_{0.05} = 2.9 µg CH₄-C m⁻² h⁻¹) F4 (Fig. 4).

Carbon Dioxide

During our study, a sampling date × treatment interaction ($P \le 0.0001$) was observed for CO₂-C emissions (Table 2). The pattern of CO₂-C emissions for the duration of this study was similar to that of N₂O-N emissions. However, only four of the 23 sampling dates showed no significant differences between CO₂-C emissions from control and ant influenced soils

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370 (LSD_{0.05} = 2.3 mg CO_2 -C m⁻² h⁻¹). When differences were observed, ant 371 influenced soil emitted more CO₂-C than control soil on 13 sampling dates, 372 whereas control soil emitted more CO₂-C, as compared to ant influenced soil, 373 on only six sampling dates (Fig. 1).

The mean flux of CO_2 -C across all sampling dates was 11 and 20 mg CO_2 -C m⁻²h⁻¹ for control and ant influenced soils, respectively. Much of the cumulative emissions observed from ant influenced soil was explained during the period between April 20 and July 28. The mean rate of emission from ant influenced soil during this period was 48 mg CO₂-C m⁻²h⁻¹ per sampling date. Following July 28, the mean rate of emission of CO₂-C dropped to 7 mg CO₂-C m⁻²h⁻¹.

Similarly, much of the cumulative emissions observed from control soil also occurred during the period between April 20 and July 28. During this period, the mean rate of CO₂-C emission was 15 mg CO_2 -C m⁻² h⁻¹; nearly double the mean rate of emission observed after July 28.

In addition to CO_2 -C emissions, soil C was another index of ant activity observed during this study. For soil C, a significant sampling date × depth × treatment interaction (P ≤ 0.0001) was observed (Table 3). Within each sampling date, soil C decreased with depth for both treatments, however, there appeared to be greater loss of soil C from ant influenced soil as compared to control soil. Mean soil C concentrations, across all depths and sampling dates, was 9.9 and 7.8 g C kg soil⁻¹ for control and ant influenced soils, respectively.

DISCUSSION

Nitrous Oxide

The mean N₂O-N emission rate observed from control soil (8.6 μ g N₂O-N m⁻²h⁻¹) was similar to the mean emission rate of 10 μ g N₂O-N m⁻²h⁻¹ from an unfertilized pasture reported by Mosier and Schimel,^[18] and within the range of emissions reported by Ambus and Christensen^[19] from an unfertilized coastal grassland. However, rates of N₂O-N emission from ant influenced soil often exceeded peak rate values shown in these studies, and were more analogous to rates seen following soil applications of highly mineralizable organic wastes.^[20,21]

It also appeared there was a seasonal influence on N₂O-N emissions for both control and ant influenced soils. Between the period of May 19 and July F5 14, temperatures ranged from 23°C to 33°C (Fig. 5). This temperature range was within the optimum range for nitrification, as well as denitrification.^[22,23] Soil moisture content was between 90–180 g water kg dried soil⁻¹ for

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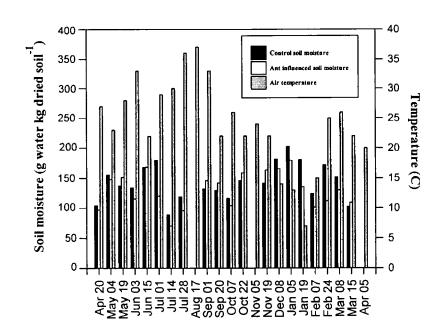


Figure 5. Air temperature, and soil moisture for control and fire ant plots (0-20 cm) near Auburn, AL from April 1999 to April 2000.

control soil, and 80-150 g water kg dried soil⁻¹ for ant influenced soil from a 0-20 cm depth.

Increased emission rates during the summer months, when soil retains adequate moisture, has been reported by others.^[9,24,25] In unfertilized soil, Bremner et al.^[24] found 55–83% of the annual emission of N₂O-N occurred between the summer months of June and August. However, it was concluded that temperature and soil water content could not explain all of the seasonal variation, and that nitrifiable N content also played a role in this seasonal flux.

442 The content of nitrifiable N may explain the large flux of N_2O -N observed 443 in ant influenced soil during the period of May 19 and July 14. It is possible 444 that high amounts of organic materials brought into the mound by foragers 445 may have increased nitrifiable N. Red imported fire ants utilize a wide variety 446 of animal, plant, fungal, and bacterial food.^[26] In warm months, fire ants favor 447 proteins over carbohydrates,^[27] and during these warmer months, the average 448 colony consumes nearly 454 g of insects and sugar a week.^[28]

449 Higher concentrations of soil NO_3 -N and NH_4 -N to a depth of 60 cm were 450 found in ant influenced soil, and it has been reported by others that 451 higher concentrations of both NO_3 -N and NH_4 -N can increase emissions

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452 of N₂O-N.^[29,30] The soil NO₃-N concentration that yields maximum rates of 453 N₂O-N emission via denitrification is variable, however, Limmer and Steele^[30] 454 reported the maximum denitrification rate for a range of soils occurred when the 455 NO₃-N concentration was approximately 25 mg NO_3 -N kg soil⁻¹. This 456 concentration value was close to the mean concentration of NO₃-N found 457 across all depths in ant influenced soil (23.6 mg NO₃-N kg soil⁻¹).

Higher concentrations of NH₄-N may also stimulate N₂O-N emissions by 458 increasing nitrification. Mosier et al.^[29] found that for cropped and native 459 soils, emissions of N2O-N increased with concentrations of NH4-N between 1 460 and 6 mg NH₄-N kg soil⁻¹. Emissions of N₂O-N from nitrification became 461 constant when NH₄-N concentrations exceeded 10 mg NH_4 -N kg soil⁻¹. At a 462 mean concentration of 18.5 mg NH₄-N kg soil⁻¹ for ant influenced soil across 463 all depths, it is likely that significant contributions to N₂O-N emissions were 464 made via nitrification. Another indicator that considerable nitrification was 465 occurring was the lower soil pH value observed from ant influenced soil, as 466 compared to control soil. 467

Soil pH may also influence N₂O-N emissions from soil. However, this 468 influence is often complex, and its effects may be unclear. When soil pH 469 ranges from <5 to 6, N₂O-N emissions tend to decrease with increasing 470 pH when denitrification is the main source of N₂O-N.^[9] However, when soil 471 pH ranges from 7 to 8, denitrification increases with pH.^[31] When nitrification 472 is the main source of N2O-N, emissions tend to increase with increasing pH 473 from a range of 6 to 8. However, in acid soils (up to pH 5), autotrophic 474 nitrification decreases with increasing pH.^[9] The mean soil pH of control soil 475 during our study was 5.9, whereas the mean soil pH for ant influenced soil was 476 5.6. Though both of these pH values were slightly less than that required for 477 optimum denitrification and nitrification, and while soil pH was shown to be 478 significantly different between treatments, it is doubtful there was enough 479 difference in pH to account for differences in N2O-N emissions observed 480 between control and ant influenced soils. 481

It is possible that the seasonal fluxes observed between May 19 and July 482 14 were associated with activities relating to the brood production or even 483 preparation for mating (nuptial) flights. While broods of red imported fire ants 484 are produced year around in warmer regions of the U.S., Lofgren et al.^[32] 485 reported May as the peak month for brood production. It is possible that the 486 higher emissions seen in late spring and early summer are related higher brood 487 production. Another possibility may be mating flights. While mating flights 488 can occur between spring and fall in the U.S., the peak months for this 489 behavior occur at the same time as our observed peak emission rates.^[26] 490 However, without further investigation, definite correlation between these ant 491 colony behaviors and increased N2O-N emissions cannot be made. 492

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Methane

Though CH₄-C emissions were found to be highly variable during this study, there seemed to be evidence that ants influence CH₄-C uptake and 496 emission. Increased net CH₄-C emission $(3.5 \,\mu g \, N_2 O - N \, m^{-2} \, h^{-1})$ by ant 497 influenced soil may be due to higher NH4-N concentrations observed in ant 498 influenced soil. Tlustos et al.^[33] reported CH₄-C oxidation decreased in 499 inverse proportion to the amount of N added as fertilizer. This was supported 500 by Hütsch,^[34] who found when NH₄CL, (NH₄)₂SO₄, and urea were applied to 501 a silty loam soil at a rate of 40 mg N kg soil⁻¹, oxidation of CH₄-C decreased 502 by 96%, 80%, and 84%, respectively, soon after application. Across all 503 sampling dates for ant influenced soil, the concentration of NH₄-N from 0 to 504 100 cm ranged from 4.4 to $32.0 \text{ mg NH}_4\text{-}N \text{ kg soil}^{-1}$, and averaged 505 18.5 mg NH₄-N kg soil⁻¹ across all depths. Ammonium found in these 506 concentrations likely had a profound influence on the ability of ant influenced 507 soil to oxidize CH₄-C. 508

Another possibility for increased net CH_4 -C emission by ant influenced soil may be related to the biological function of the ants themselves. It has been shown that termite associated CH_4 -C is produced by symbiotic microorganisms found in the hindgut.^[11,12] However, it cannot be concluded that this was the case for the red imported fire ant. More research would have to be directed toward the study of digestive functions of this species of insect to determine if CH_4 -C emission is a by-product of their metabolism.

Carbon Dioxide

Respiration by ants and bacteria within the colonies likely accounted for 520 the greater CO₂-C emissions observed from ant influenced soil, as compared 521 to control soil. Surface emission rates of CO₂-C during the spring and summer 522 were often three to five times greater from ant influenced soil than control soil, 523 and the overall mean of CO2-C emission rates throughout the study from ant 524 influenced soil was double that from control soil. The substantially higher 525 rates of CO₂-C emissions from ant influenced soil was thought to be due, not 526 only to respiration by the ants, but also to increased microbial activity within 527 the colonies. Czerwiński et al.^[6] reported greater population sizes of bacteria 528 and fungi within ant hills as compared to non-ant influenced meadow soil. The 529 development of these bacterial and fungal communities, as well as their 530 increased activity, was a response to the highly mineralizable organic matter 531 deposited in the nests by ants. It was concluded by Dauber and Wolters^[13] that 532 533 this increase in microbial activity within the mound was primarily responsible

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for increases observed in C mineralization. Dauber and Wolters^[13] reported that CO₂-C evolution from the mound soil of three ant species was between 1.7 and 2.7 times greater than that from non-ant influenced soil. Similarly, Lenoir et al.^[4] reported that when soil moisture was sufficient enough to stimulate microbial activity (60% water holding capacity), CO₂-C evolution rates were as high as 1.5 times greater than non-ant influenced forest floor soil.

Estimation of Greenhouse Gas Flux

To determine the influence red imported fire ants have on greenhouse gas 544 fluxes from soil, an estimate was made of the amount of land affected by their 545 colonies. From surveys conducted between April 1999 and April 2000, it was 546 estimated that an average of 49 fire ant mounds ha^{-1} existed on this pasture 547 throughout the year. While mound densities vary greatly throughout the 548 southern U.S., this estimation was lower than numbers of red imported fire ant 549 mounds reported by Porter et al.,^[35] Diffie and Bass,^[36] and Macom and 550 Porter.^[28] The average area of ant mounds surveyed in our study was 551 $0.12 \text{ m}^2 \text{ mound}^{-1}$, which was similar to the mean area $(0.14 \text{ m}^2 \text{ mound}^{-1})$ 552 reported by Porter et al.^[35] From these estimates, it was calculated that ant 553 influenced soil comprised 6.1 m² of each ha of land. 554

The calculated annual flux of N₂O-N from this Piedmont Plateau bermudagrass pasture, without emissions from fire ant mounds, was $7.0 \text{ kg N}_2\text{O-N ha}^{-1}$. When emissions from fire ant mounds were considered, it was estimated that an additional $6.95 \text{ g N}_2\text{O-N ha}^{-1}$ was supplied. Ant influenced soil increased N₂O-N emissions from this bermudagrass pasture by 1.0%.

A discernable influence by fire ants on CH₄-C flux was most difficult to 561 detect. When annual flux of CH₄-C was calculated, it was determined that this 562 Piedmont Plateau bermudagrass pasture oxidized 0.58 kg CH_4 -C ha⁻¹. When 563 the influence of fire ant mound emissions was taken into account, it was 564 concluded that only an additional 160 mg CH₄-C was emitted per ha. While 565 ant influenced soil was estimated to have decreased CH₄-C oxidation by 566 0.03%, this change in CH₄-C flux was rather insignificant, and was likely 567 568 within our error of measurement.

The estimated annual flux of CO_2 -C from this Piedmont Plateau bermudagrass pasture, without emissions from fire ant mounds, was calculated to be 821.9 kg CO₂-C ha⁻¹. When emissions from fire ant mounds were considered, it was estimated that an additional 0.9 kg CO₂-C ha⁻¹ was supplied. Ant influenced soil increased CO₂-C emissions from this bermudagrass pasture by only 0.1%.

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575 It can be speculated that regional and global budgets of N_2O-N , CH_4-C , 576 and CO_2-C emissions may be underestimated when the influence of soil 577 macro-organisms, such ants, are not taken into account. However, the 578 underestimation may only be slight given the data presented here.

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