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Ants Nest and Its Association in Soil Headspace Ethylene Concentration

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Abstract
Ants are recognized as ecosystem engineers because they modify soil structure and nutrient composition through nest construction and associated activities. Information on the influence of ant nest on soil headspace ethylene concentration remains unattended. This study aimed to compare the physicochemical properties of ant nest soil with surrounding soil and to evaluate their relationship with headspace ethylene concentration. Soil samples were collected from Kunnathur Village, Tirunelveli, Tamil Nadu, India, comprising 10 ant nest soil samples and 10 surrounding soil samples. Soil physicochemical properties were determined using standard laboratory methods, and headspace ethylene was analysed by gas chromatography. The data were analysed using an independent *t*-test, one-way analysis of variance (ANOVA) followed by Tukey's HSD test, and Pearson's correlation analysis. Ant nest soil exhibited significantly higher organic carbon, nitrogen, iron, copper, and boron contents and a significantly lower, near-neutral pH than the surrounding soil ($P < 0.05$). One-way ANOVA and Tukey's HSD test showed that headspace ethylene concentration was significantly lower in ant nest soil than in surrounding soil ($P < 0.05$); whereas, no significant differences were observed between ant nest species ($P > 0.05$). Pearson's correlation analysis revealed that organic carbon($r=-0.687$, $p=0.0041$), nitrogen($r=-0.883$, $p=0.0016$), sulphur($r=0.676$, $p=0.046$), and copper($r=-0.713$, $p=0.031$) were significantly associated with headspace ethylene concentration. This finding indicated that ant nesting activity alters soil physicochemical properties, which may influence ethylene behaviour in soil. This study improves our understanding about the relationship between ant-mediated soil modification and soil gas dynamics.

Introduction

Soil is a complex system interacting among the lithosphere, atmosphere, hydrosphere, and biosphere. In other words, it is the interface where the interaction occurs (Usharani KV, 2019). The overall soil nature and quality are fundamentally important to ecosystems (Pal, 2014). Soil is a habitat for many terrestrial animals that contribute to the modification of the soil's physical, chemical, and biological properties. In terrestrial ecosystems, social insects are regarded as one of the most diverse and widely distributed groups (Folgrait 1998; Frouz and Jilkova 2008).

In soil ecosystem, ants are considered the most successful social insects in terms of number, biomass, and distribution (Holldobler & Wilson 1990). Ants use soil to construct their nests, bring materials to the surface, and add components such as food and organic materials, which enrich the nutrient levels, thus altering soil properties and the ecological functioning of the habitat; hence, they are referred to as ecosystem engineers (Holldobler & Wilson 1990, Whitford 2013, Folgarait 1998, Wu et al. 2015). Ant nest soil has higher concentrations of organic matter, nitrogen, phosphorus, potassium, and several micronutrients than the surrounding soil, making them better for plants to grow by improving soil fertility and biological quality (Wagner et al., 2004; Farji-Brener & Werenkraut, 2017). The interconnected chambers and tunnels that control the flow of air, through its structure, soil properties, dynamics, and activity of the ants (Holldobler & Wilson, 1990).

Ethylene (C2H4), a plant hormone, is produced by plant roots and soil microorganisms and is influenced by environmental and physicochemical factors. Soil moisture, nitrate availability, and physical conditions affect ethylene behaviour in soils (Van Cleemput et al., 1983). Aeration, microbial activity, and moisture content affect ethylene occurrence in soils (Lang et al., 2023). Adsorption of ethylene also varies with soil properties, such as clay mineral composition and pH (Witt & Weber, 1975). This highlights the importance of soil physicochemical properties in controlling ethylene dynamics in soil.

As well, ant nests have significant and varied effects on the physicochemical properties of the soil, and these qualities influence the behaviour of gases in the soil. However, knowledge regarding the influence of ant nests on ethylene production is limited. This study aimed to focus on the influence of physicochemical properties of ant nest soil on the concentration of headspace ethylene. Therefore, this study focused on (i) comparing the physicochemical properties of ant nest soil and surrounding soils, (ii) evaluating differences in headspace ethylene concentrations using gas chromatography, and (iii) determining the associations between physicochemical properties and ethylene concentrations. This study provided a better understanding of the modifications in soil induced by ants and ethylene, and contributes to a deeper comprehension of the dynamics of gas in the soil.

Materials & Methods

Sample collection

Soil samples were collected from Kunnathur village in Tirunelveli, Tamil Nadu, India. Ten ant nest soil samples and ten surrounding soil samples were collected from different locations, including near houses, agricultural fields, and near tree roots where ant nests were naturally present. Soil expelled by ants and deposited around the nest entrance was collected as an ant-nest soil sample. Normal soil was collected two meters away and used as a control. The debris, plant roots, and dried leaves were removed from the collected soil and transferred into a zip-lock cover, and the sample name were noted for subsequent analysis.

Physiochemical analysis

The collected soil samples were submitted to the Tamil Nadu Government Department of Agriculture and Farmers' Welfare, Tirunelveli, Tamil Nadu, India for physicochemical analysis. Soil samples were analysed using standard laboratory procedures. Parameters including soil texture, electrical conductivity (EC), pH, organic carbon (OC), nitrogen(N), phosphorus(P), potassium(K), sulphur(S), iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), and boron (B) were determined.

Sample preparation for GC Analysis

For gas chromatography analysis, red ant nest soil (*Solenopsis geminata*) and Black ant nest soil (*Camponotus compressus*) were collected. Five grams of either normal soil or ant nest soil were placed in a conical flask fitted with a side arm for gas collection. Then, 30 mL of 5 M potassium hydroxide (KOH) solution was added to the soil, and the flask was sealed immediately. Fifty milliliters of ethephon were placed in an upper round flask. A control containing only KOH and ethephon (without soil) was prepared. The ethylene generation procedure was adapted from the method described by Sadasivam and Manickam (*Biochemical Methods*, 4th Edition, p. 229). Immediately after the ethephon was allowed to mix with the KOH–soil mixture, the generated gas was collected through the side arm using a 20 mL gas syringe and subjected to gas chromatography (GC) analysis. The same procedure was followed for the control, surrounding soil, and ant nest soil treatment groups. The experiment was performed in triplicate for each treatment, and the GC peak characteristics were compared to evaluate the influence of soil on the measured ethylene peaks

Gas Chromatography Analysis

The generated gas samples were analysed using a Shimadzu GC-2014 gas chromatograph equipped with a thermal conductivity detector (TCD) fitted with a Porapak column. Nitrogen was used as the carrier gas at a flow rate of 8 ml/min, injector temperature of 100°C, column temperature of 150°C, detector temperature of 250°C, and detector current of 60 mA. The run time was 10 min with an equilibration time of 1 min. Ethylene production was evaluated by comparing the peak areas of the different soil samples.

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Statistical Analysis

The data obtained from the study were analysed using Microsoft Excel software. An independent *t*-test was used to compare the physicochemical properties of ant nest soil and surrounding soil. Differences in GC peak characteristics among normal soil, red ant nest soil, and Black ant nest soil were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's HSD test for multiple comparisons. Pearson's correlation analysis was performed to determine the relationship between soil physicochemical properties and GC peak characteristics. A *p*-value of less than 0.05 was considered statistically significant

Results

Table 1: The physical properties of two different soils

Soil Texture	Ant Nest Soil (n=10)	Normal soil (n=10)
Sandy Clay Loam	8	2
Sandy Loam	2	5
Sandy	0	3

Among the 20 soil samples analyzed, ant nest soil was classified as 8 sandy clay loams and 2 sandy loams. The normal soil comprises 2 sandy clay loams, 5 sandy loams, and 3 sandy

Table 2: Statistical data of soil chemical parameters using t-test

Sl. No.	Parameters	Ant Nest Soil Mean ± SD	Normal Soil Mean ± SD	t-value	p-value	Significance
1	EC (dSm^{-1})	0.523±0.491	0.457±0.417	0.323	0.750	NS
2	pH	7.28±0.913	8.23±0.326	-3.095	0.01	**S**
3	OC (%)	0.64 ±0.314	0.37±0.240	2.188	0.04	**S**
4	N (Kg/acre)	81.1±16.676	53.4±17.727	3.599	0.002	**S**
5	P (Kg/acre)	3.7±2.869	4±6.765	-0.129	0.899	NS
6	K (Kg/acre)	135.2±67.053	123.3±139.01	0.243	0.811	NS
7	S (Kg/acre)	8±11.869	18±26.985	-1.072	0.304	NS
8	Fe (ppm)	28.012±26.897	7.606±5.909	2.343	0.04	**S**
9	Mn (ppm)	14.088±6.541	47.75±131.258	-0.810	0.438	NS
10	Zn (ppm)	1.911±2.376	0.904±0.609	1.296	0.223	NS
11	Cu (ppm)	1.759±0.854	0.708±0.326	3.633	0.003	**S**
12	B (ppm)	0.491±0.209	0.3±0.12	2.499	0.02	**S**

Note: S = Significant at 5% level; NS = Not Significant

Soil Chemical Parameters

Table 2 presents a comparative statistical analysis of chemical parameters between ant nest soil and normal soil. The data revealed significant differences in pH, Organic Carbon, Nitrogen, Iron, copper, and Boron.

The pH of the ant nest soil (7.28 ± 0.913) was significantly lower indicating near-neutral pH than that of the normal soil (8.23 ± 0.326; t = -3.095, p = 0.01), and organic carbon of nest soil was significantly higher compared to normal soil (OC: 0.64 ± 0.314% vs. 0.37 ± 0.240%; t = 2.188, p = 0.04). Similarly, ant nest soil showed significantly enriched macronutrient such as nitrogen (N: 81.1 ± 16.676 vs. 53.4 ± 17.727; t = 3.599, p = 0.002) and micronutrients such as Iron (Fe: 28.012 ± 26.897 vs. 7.606 ± 5.909; t = 2.343, p = 0.04), Copper (Cu: 1.759 ± 0.854 vs. 0.708 ± 0.326; t = 3.633, p = 0.003), and Boron (B: 0.491 ± 0.209 vs. 0.3 ± 0.12; t = 2.499, p = 0.02).

In contrast, no statistically significant differences (p > 0.05) were observed between ant nest and normal soils for electrical conductivity (EC), phosphorus (P), potassium (K), sulfur (S), manganese (Mn), and zinc (Zn).

Figure 1: Physicochemical parameters of ant nest soil and surrounding soil

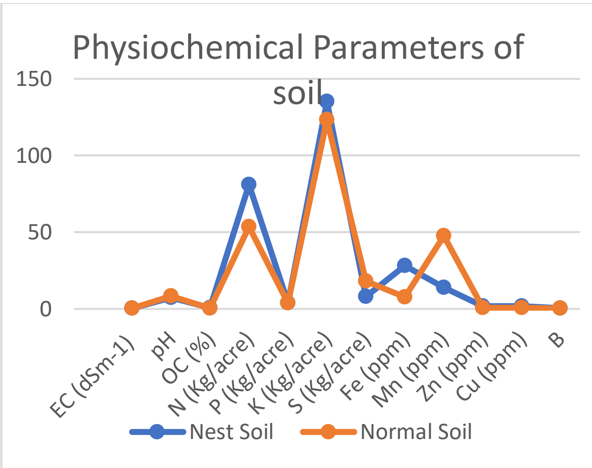

Table 3: Statistical comparison (ONE-WAY ANOVA) of Gc peak characteristics among different soil samples

Sl. No.	Parameters	Normal Soil (Mean±SD)	Red Ant Nest Soil (Mean±SD)	Black Ant Nest Soil (Mean±SD)	F-value	p-value	Significance
1	Height	195429± 37872.8	83052± 4283.66	92673.00± 31670	22.351	0.00166	**S**
2	Area	5593± 926.557	2592± 99.594	2823.67± 813.64	22.760	0.00158	**S**
3	Area %	27.707± 3.876	14.016± 0.936	15.22± 4.25	23.886	0.00139	**S**

Note: S = Significant at 5% level; NS = Not Significant

One-way ANOVA showed a significant difference in the GC parameters among normal soil, red ant, and Black ant nest soil. Significant difference was observed for peak height (F=22.357, p=0.001), peak area (F=22.760, p=0.001), and area percentage (F=23.886, p=0.001)

Figure 2: CONTROL (KOH + Ethephon)

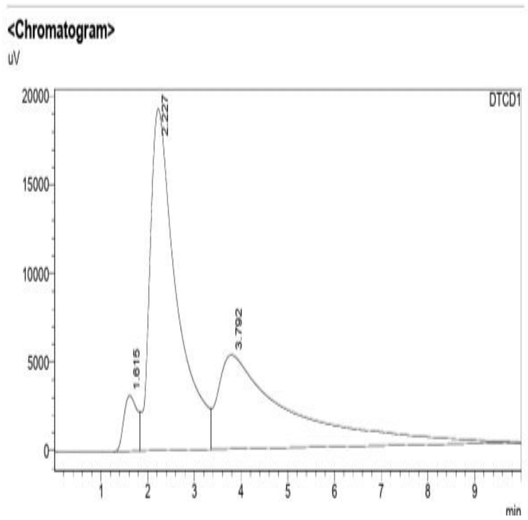

Figure 3: Normal Soil 01,

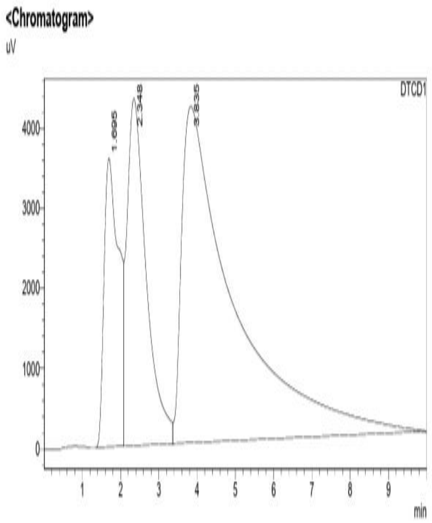

Figure 4: Normal Soil 02

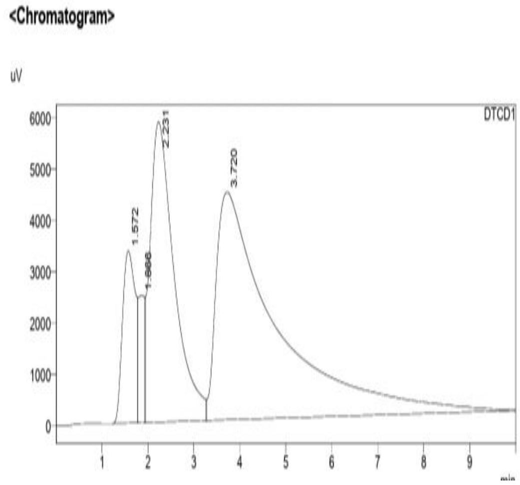

Figure 5: Normal Soil 03

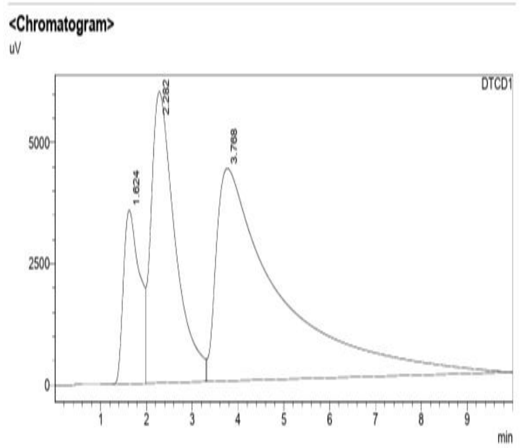

Figure 6: Red Ant Soil 01

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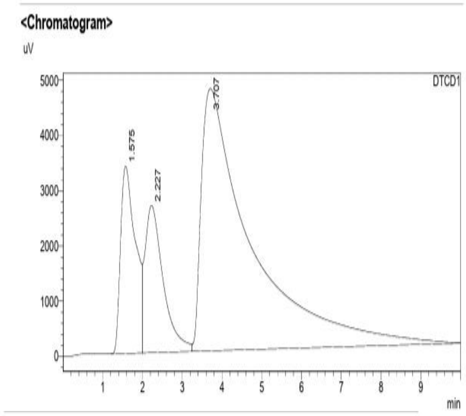

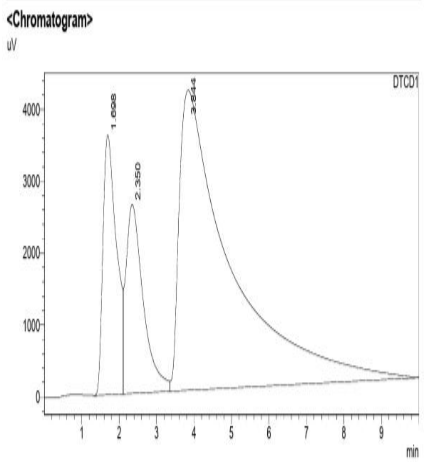

Figure 7: Red Ant Soil 02

Figure 8: Red Ant Soil 03

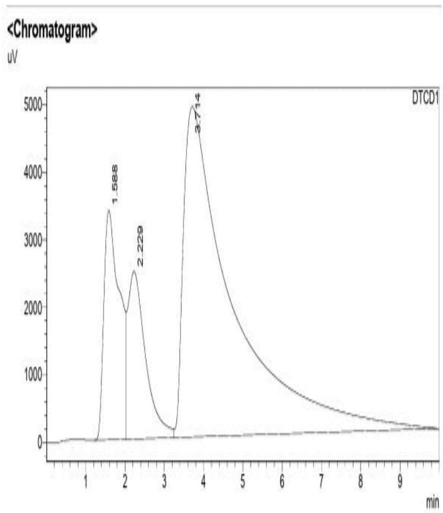

Figure 9: Black Ant Soil 01

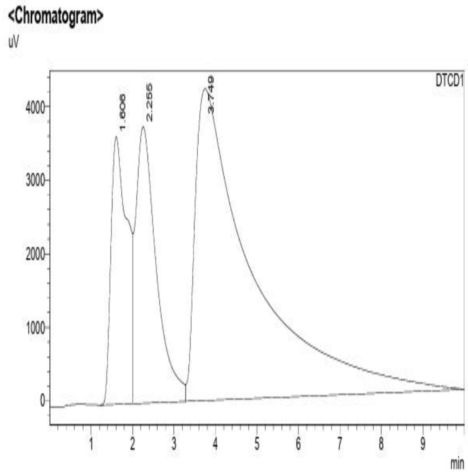

Figure 10: Black Ant Soil 02

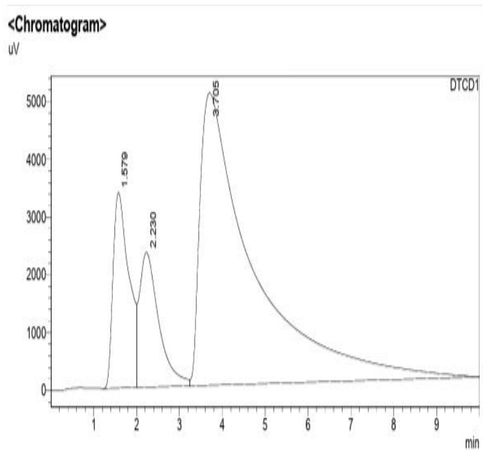

Figure 11: Black Ant Soil 03

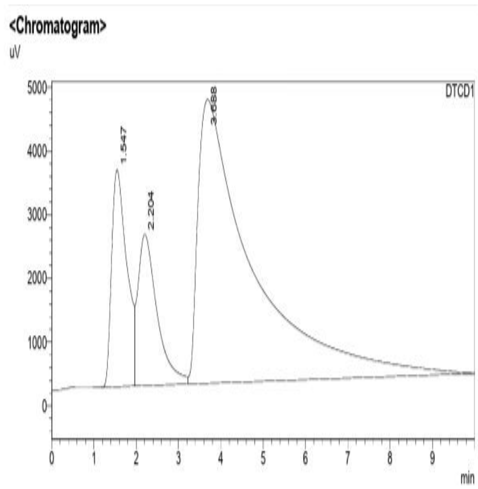

Table 4: Tukey HSD test for Gc parameters

Characteristics	Pair	Difference	P - value	Significance
Height	X1 – X2	112377.3333	0.007065	**S**
	X1 – X3	102689.6666	0.01084	**S**
	X2 – X3	9687.6667	0.9108	NS
Area	X1 – X2	2801	0.007134	**S**
	X1 – X3	2569.3333	0.01075	**S**
	X2 – X3	231.6667	0.9178	NS
Area %	X1 – X2	13.691	0.006096	**S**
	X1 – X3	12.4883	0.009468	**S**
	X2 – X3	1.2027	0.9022	NS

Note: S = Significant at 5% level; NS = Not Significant

The Tukey HSD test indicated that normal soil differed significantly from both red ant nest soil and black ant nest soil for all GC parameters. However, no significant difference was observed between red ant nest soil and black ant nest soil. These findings indicate that the two nest soils were similar to each other but differed significantly from normal soil.

Table 5: Pearson correlation between ethylene production and soil physicochemical properties

PROPERTY	Pearson's Correlation	p value
EC (dSm^{-1})	0.454	0.220
pH	0.496	0.175
OC (%)	-0.687	0.041
N (Kg/acre)	-0.883	0.0016
P (Kg/acre)	-0.557	0.119
K (Kg/acre)	-0.612	0.080
S (Kg/acre)	0.676	0.046
Fe (ppm)	-0.585	0.098
Mn (ppm)	-0.274	0.475
Zn (ppm)	-0.377	0.318
Cu (ppm)	-0.713	0.031
B (ppm)	-0.194	0.618

Note: S = Significant at 5% level; NS = Not Significant

Pearson correlation analysis revealed that organic carbon(r=-0.687, p=0.0041), nitrogen(r=-0.883, p=0.0016), sulphur(r=0.676, p=0.046), and copper(r=-0.713, p=0.031) were significantly correlated with ethylene production ($p < 0.05$); whereas, the remaining soil physicochemical properties were not ($p > 0.05$).

Discussion

The present study demonstrated that the headspace ethylene concentration in ant nest soil is significantly lower compared to surrounding soil; one-way ANOVA confirms this, and Tukey's HSD post hoc test showed significant differences in surrounding soil and both ant nest soils; however, no significant differences were observed between the two nest soils. Witt & Weber (1975) stated that ethylene movement shows variation in different soil properties; greater retention capacity may reduce ethylene mobility and diffusion. In the current study, the occurrence of lower ethylene headspace concentration in ant nest soil may be due to its significant physicochemical properties. Correlation analysis indicated that organic carbon (OC), nitrogen (N), sulfur (S), and Copper (Cu) were significantly associated with ethylene concentration. Frouz & Jílková (2008) and Farji-Brener & Werenkraut (2017) studies showed that nest creation can modify soil properties, including organic matter accumulation and nutrient availability, which support our present study. Ant activity, such as deposition of food, accumulation of seeds, plant debris, dead insects, and other activities, increases the organic material inside the nest, contributing to higher organic carbon. Deposition of accumulated organic material and ant waste enriches the nitrogen content (Folgarait, 1998; Frouz & Jílková, 2008). To build the nest, ants excavate soil from deeper layers to the surface and redistribute soil through activities like digging, burrowing, and tunnelling. Through excavation and soil redistribution, ants can alter the spatial distribution of soil minerals and nutrients, potentially influencing micronutrient availability (Frouz & Jílková, 2008). In the present study, higher OC, N, Fe, Cu, and B concentrations were observed in ant nest soils. The accumulation of nest refuse and organic materials may have contributed to the near-neutral pH observed in ant nest soil by modifying soil chemical properties (Farji-Brener & Werenkraut, 2017). Ants also modify soil texture through excavation, particle redistribution, and nest construction (Cammeraat & Risch, 2008), in current study the nest soil texture varied from surrounding soil. Overall, the enrichment of organic carbon, nitrogen, iron, copper, and boron, together with the near-neutral pH of ant nest soil, may have influenced ethylene retention and movement thereby contributing to the lower headspace ethylene concentration observed in the present study.

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