

Effect of Naphthalene on Soil Dynamics in Order to Tackle Soil Micro-Arthropods

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Abstract: For many years, decomposition tests have employed naphthalene, a polycyclic aromatic hydrocarbon (C₁₀H₈), to reduce soil fauna. This work addresses the effectiveness of naphthalene additives for suppressing soil fauna and possible off-target impacts on the soil microbial community and carbon cycling in the field. Naphthalene was applied at a high rate (475 gm/m²) once a month to the exposed soil surface of alluvial soil. At 10, 15, and 20 months following the start of naphthalene application, we assessed the impact of such additions on the abundance of nematodes and micro-arthropods along the soil profile to a depth of 20 cm. To measure the contribution of naphthalene to soil CO₂ efflux and microbiological biomarkers Phospho Lipid Fatty Acid (PLFA) analysis method was used. We used the variance in the natural ¹³C abundance of naphthalene (δ¹³C – 24.5‰) as compared to the native soil (δ¹³C ~–16.5‰). The addition of naphthalene markedly decreased the number of springtails (–38%), predatory mites (–49%), and oribatid mites (–35%), but had no effect on the number of nematodes. A few Gram-positive (a14:0, i14:0), Gram-negative (cy16:0, 17:1ω7c, 15:1ω7c), and Actinobacteria (10Me-15:0, 10Me-17:0) PLFA markers showed a significant drop in ¹³C abundance in plots treated with naphthalene, suggesting that the bacteria were using the naphthalene-derived C. This experiment demonstrates that adding naphthalene to soil can effectively decrease soil micro-arthropods in the field, with few direct effects on microbial abundance, C dynamics, or soil nematodes.

Keywords — Polycyclic Aromatic Hydrocarbon, Nematodes, Micro Arthropods, CO₂ efflux, PLFA.

I. INTRODUCTION

It is widely recognized that the actions of the bigger soil fauna have a considerable impact on nutrient cycling, even though microbes govern most soil biogeochemical processes [1]. For instance, a lot of soil fauna eat litter that has been conditioned by microorganisms or feed on the microbes responsible for breaking down litter [2]. Thus, an explicit knowledge of the structure of the soil food web and the interactions among the soil biota within it is necessary to effectively predict biogeochemical cycling [3]. As naphthalene, a polycyclic aromatic hydrocarbon (C₁₀H₈), has been shown to suppress soil invertebrate abundances with limited non target effects when applied in comparison to other biocides [4], it has long been used to evaluate the role of soil fauna in litter decomposition processes. Such preliminary research [5] revealed that soil fauna can strongly accelerate decomposition rates. The decomposition of *Drypetes glauca* and *Cedraia odorata*, but not *Rhododendron maxima*, is greatly aided by soil fauna, according to a study by Heneghan et al. (1998), indicating that the role of soil fauna is influenced by litter quality.

According to Wang et al. (2009), soil fauna has a less impact on the decomposition of litter at higher elevations, indicating the influence of climate. Similarly, soil fauna accelerates decomposition rates in temperate and moist tropical habitats, but not in ecosystems where biological activity is limited by climatic conditions, according to a worldwide litter decomposition study conducted by Wall et al. (2008). These investigations bolster the value of micro-arthropod suppression in ecological research.

Though widely used, it is still uncertain if suppressing soil fauna with naphthalene is appropriate in investigations that look into the role of soil fauna in litter and soil biogeochemical processes. Microbes are able to metabolise naphthalene [6]. Although volatilisation can swiftly eliminate naphthalene from the soil [7], adsorbed on soil organic materials may lengthen naphthalene's survival in soils. According to [14], the amount of organic matter in the soil determines its sorption capacity, which is also influenced diffusively by the texture and water content of the soil [8]. Naphthalene cannot be abiotically converted into CO₂ and is essentially insoluble in water. Surface applications of naphthalene may have an impact on soil

biogeochemistry, which in turn may change microbial biomass.

The objectives of this study were to: (1) determine whether naphthalene additions affect soil fauna, particularly micro-arthropods and nematodes, throughout a 23-month field incubation period; and (2) confirm whether naphthalene contributes to soil CO₂ outflow. The study was intended to test the following hypotheses in particular: (I) the addition of surface naphthalene reduces the abundance of soil micro-arthropods near the soil surface but not at depth; and (II) naphthalene has no effect on nematodes because it acts as a fumigant on the soil pore spaces where micro-arthropods live but not on the water films surrounding soil particles where nematodes reside.

The effects of naphthalene addition on soil fauna abundance, soil CO₂ efflux, and microbial community were evaluated on bare soil, following litter removal, in order to better identify the direct effect of naphthalene and distinguish it from the indirect effect caused by the soil fauna suppression of surface litter decomposition. Using the isotopic mixing model [9], [15] the natural ¹³C abundance differences between naphthalene ($\delta^{13}\text{C} - 24.5\%$) and background soil respiration ($\delta^{13}\text{C} - 15.7\%$) and microbial phospholipid fatty acids (PLFA; $\delta^{13}\text{C} \sim -16.5\%$) were used to determine naphthalene's contribution to soil CO₂ efflux and microbial biomass.

II. MATERIALS AND METHODS

A. Experimental Design

At the Dream Institute of Technology's ecological research facility in Kolkata, the study was carried out. The predominant plant on the tall grass pampa, *Phalaris Arundinacea*, is abundant. There is an average annual temperature of 26.8 °C and 1836 mm of precipitation at this tropical savanna habitat. Between 2.06% C and 0.40% N in the top 20 cm of the soil, the soil is silt-clay-loam alluvial.

Two naphthalene addition rates—without naphthalene (control; C) and with naphthalene (475 gm/m²/month; N)—as well as three damaging harvests—10 (H1), 15 (H2), and 20 (H3) months after the naphthalene addition started included the naphthalene manipulation experiment. In order to simulate sample dates of a typical medium-term field decomposition experiment, harvest times were used.

A PVC collar (20 cm in diameter and 10 cm in height) that was buried 5 cm in the ground served as the experimental unit. The experiment is set up with 4 replicate blocks. 2 PVC collars (subplots) are present in each complete plot; one is treated with naphthalene, while the other one acts as a control. Different depths of soil were sampled for every PVC collar.

B. Soil CO₂ Efflux

Using a 20 cm diameter survey chamber and a portable infrared gas analyzer (LI-8100), soil CO₂ efflux was recorded on a regular basis. The LI-8100 program was used to calculate the rate of CO₂ outflow. To reach a CO₂ concentration range suitable for applying the Keeling plot method for measuring $\delta^{13}\text{C}$ of soil CO₂ efflux, the chamber was closed for a total of 600 s [10]. After the gas was collected in the field, the LI-8100 software was used to determine the CO₂ concentration of the gas samples.

C. Fauna Extraction

A subsample of the main soil sample was used to remove nematodes using the Baermann funnel technique [11], [16] and [17]. Nematodes were extracted using around 100 g of fresh soil after each soil sample had been slightly homogenized. Forty and sixty grams of soil were removed from the 0–2 and 2–5 cm layers, respectively, and mixed, and one hundred grams were taken from the 5–10 and 10–20 cm layers. At 24, 48, and 72 hours, a part of 20 milliliters of deionized water and nematodes from each sample were collected in the same vial, yielding a total volume of 60 milliliters. An Olympus CKX41 inverted microscope was used to count the 5 ml remaining volume following extraction.

D. PLFA Extraction and C-PLFA measurement

Fresh litter, macro-fauna, and visible roots were eliminated from the 0–2 and 2–4 cm depth layers of the soils by screening and sieving the material down to 2 mm. A 20-gram soil subsample was lyophilised for 48 hours before being extracted using PLFA after being frozen at -20°C. Conventional procedures [12], [13] were utilised to extract phospholipid fatty acids from samples H1, H2, and H3. Free fatty acids were obtained from phospholipids by saponification, and these methylated fatty acid esters (FAMES) were then created using methanolic KOH. Using combustion isotope ratio mass spectrometry and capillary gas chromatography, FAMES were measured and examined for ¹³C.

III. RESULTS

A. Effects of Naphthalene on Soil Fauna

All nematode trophic groups were unaffected by naphthalene, and the total number of worms per kilogramme of dry soil weight varied from 579 to 20,104. Compared to Naphthalene treatment, the trophic groups of nematodes seemed to have densities that varied more with time and depth. But the only way predatory nematodes changed was with depth.

The density of all categories of micro-arthropods was significantly affected by the naphthalene treatment, in contrast to the nematodes [18]. Naphthalene considerably

decreased the number of oribatid mites represented by the Fig. 1a.

While there was no significant relationship between time and depth and oribatid mite abundance, naphthalene's effect was independent of both [19]. Naphthalene had a mostly detrimental effect on predatory mites shown in Fig. 1b. A substantial three-way interaction between sampling duration, soil depth, and naphthalene treatment explained why their densities varied. Analogously, naphthalene had a noteworthy adverse main effect on springtail density shown in the Fig. 1c.

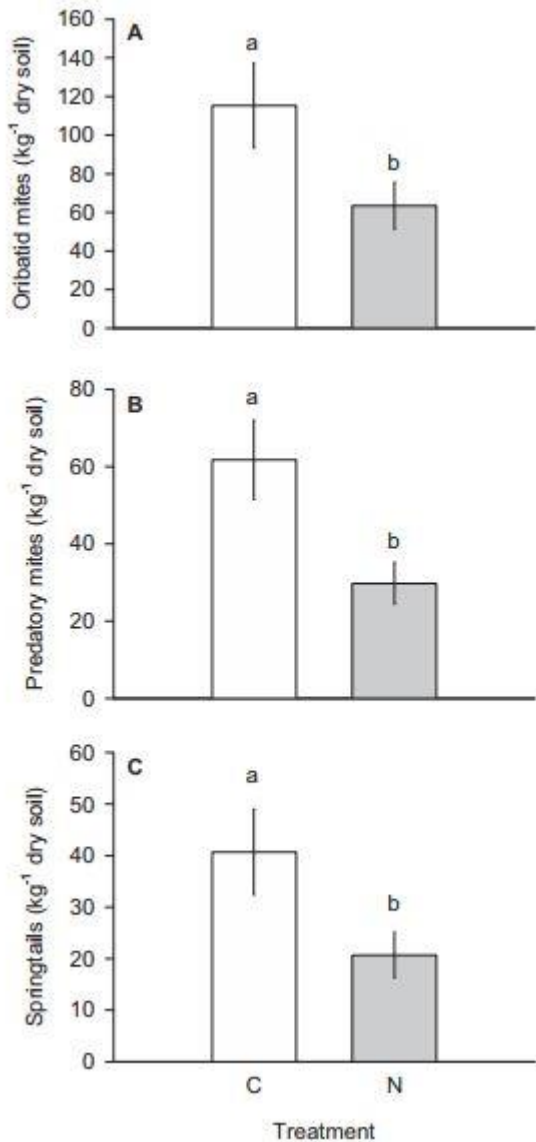


Fig. 1. Naphthalene treatment effect on (A) No. of Oribatid Mites (B) Predatory Mites and (C) Springtails. [C-Control; N-Naphthalene][14]

comparing the density of oribatid mites to the control, the population marginal mean decreased by 44%, for predatory mites it decreased by 51%, and for springtails it decreased by 48%. The naphthalene effect on oribatid mites and springtails was unaffected by the depth of the soil or the time of sampling.

B. Effects of Naphthalene on Soil CO₂ Efflux

Time-dependent variations in the total soil CO₂ outflow were observed. Fig. 2a shows that there was no significant difference in the CO₂ efflux between the naphthalene and control plots, with the former having low CO₂ efflux throughout autumn and winter and the latter have high CO₂ efflux during summer. Figure 2b shows that there was a seasonal tendency in the considerable variation of the CO₂ efflux's δ¹³C over time, suggestive of naphthalene-C having no appreciable role.

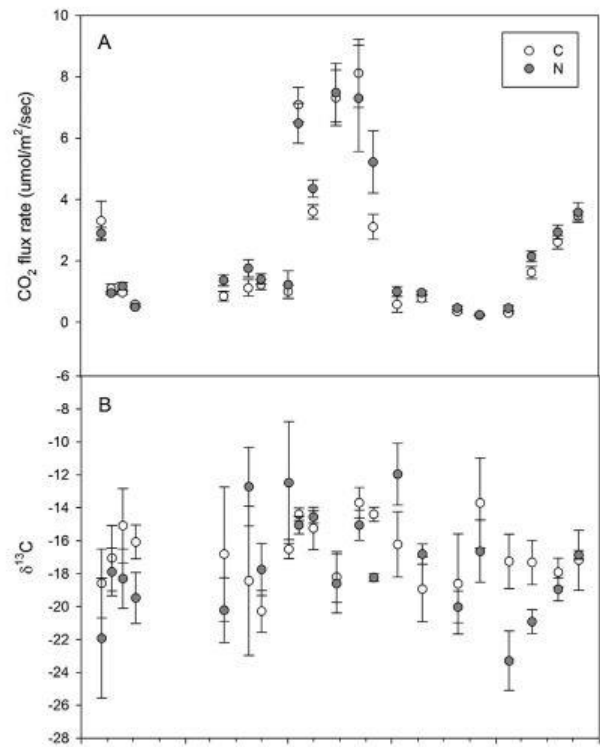


Fig. 2. The soil CO₂ efflux (A) and δ¹³C (B) for the control (C) and naphthalene treated (N) plots over the course of the experiment [14].

C. Effects of Naphthalene on microbiological PLFAs

The naphthalene treatments had no discernible effect on the quantity of PLFAs represented by Fig. 3a. Nevertheless, the ¹³C abundance of seven PLFAs was generally considerably reduced by the naphthalene treatment as shown in Fig. 3b.

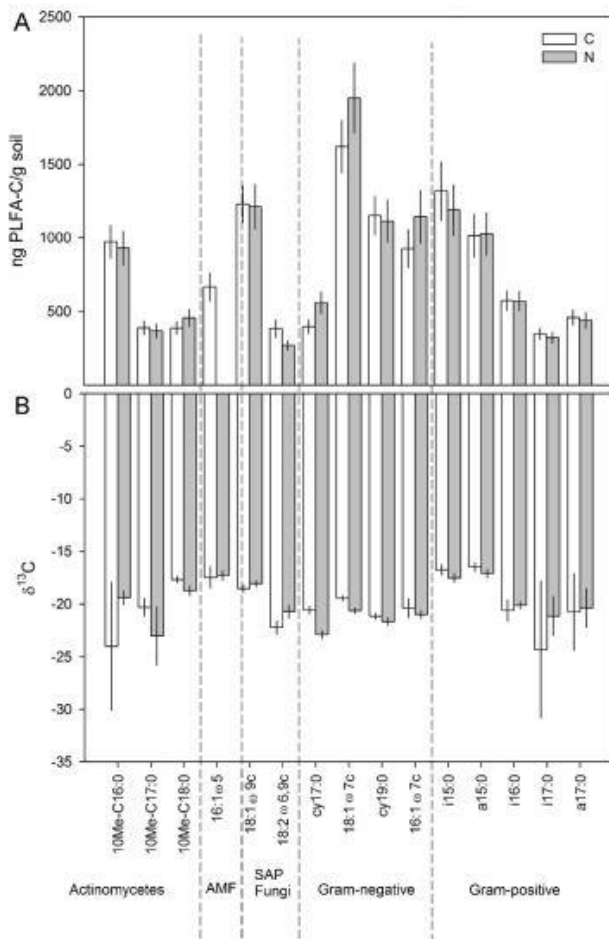


Fig. 3. The PLFA abundance (ng PLFA-C g⁻¹ soil) (A) and δ¹³C (‰) (B) for the control (C) and naphthalene treated (N) plots over the three sample dates and two depths [14].

IV. DISCUSSIONS

The Naphthalene effect on soil fauna showed the amount of soil micro-arthropods was greatly and dramatically decreased by adding naphthalene to the soil surface. On an average, this reduction was 44% for oribatid mites, 51% for predator mites, and 48% for springtails represented by Fig. 1a, Fig. 1b and Fig. 1c respectively. The fact that the effect was unaffected by soil depth suggests that adding naphthalene to the soil's surface is adequate to kill soil microarthropods down to a depth of 20 cm. Furthermore, the effects of naphthalene did not seem to change over time, suggesting that soil microarthropods are not able to adjust to its presence. The naphthalene additives did not harm nematodes. At tropical and subalpine sites with different soil moisture regimes, comparable naphthalene doses resulted in even higher arthropod suppression rates in the litter, with a reduction in the total number of arthropods ranging between 86 and 99%.

The results of the study show that naphthalene is a workable strategy for suppressing micro-arthropods and that it has no effect on non-target species like nematodes. Microbial activity is not substantially changed by the presence of naphthalene.

V. CONCLUSIONS

Therefore at the end of this study the following can be concluded:

- i. Adding naphthalene to the soil's surface is a workable way to lower the number of micro-arthropods.
- ii. The Additions are effective at depth and can be made for an extended length of time without having a noticeable negative impact on non-target variables.
- iii. While it could vary depending on the study site and conditions, the rate of suppression seems to be comparable (44–51%) among several arthropod groups within a site.
- iv. Nematodes and other types of soil fauna were not suppressed by naphthalene.

Finally, all things considered, our research points towards naphthalene addition as a viable technique for modifying the soil's arthropod population.

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