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EFFECTS OF NAPHTHALENE ON MICROBIAL ACTIVITY AND NITROGEN POOLS IN SOIL-LITTER MICROCOSMS

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Summary—Naphthalene effects on microbial respiration, numbers of bacteria and fungi, and litter and soil nitrogen pools were investigated in litter-soil microcosms containing microbes but no mesofauna. Naphthalene was applied three times during the 56 day study. Total respiration was unaffected by the first application of naphthalene, but increased upon subsequent applications. Bacteria and fungi in the litter and soil were quantified separately at the end of the study. Numbers of bacteria were significantly higher in both litter and soil of naphthalene-treated microcosms. Lengths of total and FDA-active fungal hyphae in the litter and soil, respectively, were significantly lower in the naphthalene treatment. Mass loss of litter was not affected. However, both the final concentration and absolute amount of N in the litter were reduced by naphthalene, as was soil extractable NH_4-N and NO_3- + NO_2-N. These results suggest that naphthalene may directly affect microbial populations and activity and alter nitrogen dynamics and that caution should be used in interpreting results of field studies using naphthalene to exclude microarthropods.

INTRODUCTION

The influence of soil animals on soil processes is receiving increasing attention (Coleman *et al.*, 1983; Anderson and Ineson, 1984; Seastedt, 1984). Individual studies of the influence of microarthropods on soil processes, such as litter decomposition, microbial activity and nutrient dynamics, have generally taken one of two approaches. One is the use of simplified laboratory microcosms in which the fauna can be manipulated directly. Although this approach has produced considerable evidence suggesting the importance of microarthropods in affecting soil processes, these systems do not adequately address the complexity of interactions occurring in the field. The other general approach to investigating faunal effects involves the exclusion of particular groups of interest in field experiments. Exclusion of microarthropods is usually accomplished by physical or chemical means. Both of these methods have drawbacks, however. Physical exclusion, for example by mesh size, may alter microclimate or affect the microflora (St John, 1980). Biocides have been widely used to manipulate faunal components in field studies. However, few biocides are selective and effects on non-target organisms must be considered when interpreting the results of these experiments.

Naphthalene is often used as a biocide to reduce soil and litter arthropod populations in field experiments (Witkamp and Crossley, 1966; Williams and Wiegert, 1971; Seastedt and Crossley, 1980, 1983) and is generally considered to have several advantages over other chemical biocides. It is volatile. It is water insoluble, which contributes to its immobility and persistence in the soil or litter, and it contains only carbon and hydrogen and, therefore, does not contribute exogenous nutrients. Naphthalene is very effective at reducing microarthropod numbers in the field (Seastedt and Crossley, 1980, 1983) and is generally considered to have minimal effects on non-target organisms. However, Witkamp and Crossley (1966) noted increased soil respiration and numbers of bacteria in naphthalene-treated plots. Seastedt and Crossley (1980, 1983) also commented on the potential of naphthalene to affect nutrient fluxes by stimulating microbial immobilization, thus obscuring faunal effects. More recently, Newell *et al.* (1987) reported that naphthalene vapors reduced the radial growth of fungal cultures in sealed containers. However, in field experiments (J. M. Blair *et al.*, unpublished data) we found that naphthalene did not significantly reduce the abundance of total or FDA-active fungi on litter confined in fiberglass litterbags, relative to untreated controls. In order to quantify potential direct effects of naphthalene on total microbial respiration, numbers of bacteria and fungi and nitrogen fluxes, we applied naphthalene to soil-litter microcosms containing a mix of bacteria and fungi, but no microarthropods or nematodes.

MATERIALS AND METHODS

Twenty-four replicate soil-litter microcosms were constructed in 20 dram clear plastic vials (6 cm high × 4.1 cm i.d.). Each microcosm received 20 g of air-dried soil, which was sieved (2 mm) and mixed following removal of coarse organic material. The soil was a Hiwassee sand-clay loam (Typic rhodudults) from a flood plain forest on the Georgia piedmont. Initial % ash-free dry mass (AFDM) was 2.7% and total Kjeldahl nitrogen was 920 μg g^{-1}. Circular fiberglass screens (1.6 × 1.8 mm mesh) were placed on the soil surface and 0.4 g of air-dried dogwood (*Cornus florida* L.) leaf litter (0.38 g dry wt equivalent), cut into approx. 1.5 cm pieces, was added to each microcosm. This simulated litter standing

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stocks of 300 g m^{-2}. Initial N concentration of the litter was 7.74 mg g^{-1}. Microcosms were adjusted to 30% moisture (w/w) with deionized water. Individual microcosms were microwaved in a 700 W microwave oven for 90 s and capped for 24 h at which time the microwave treatment was repeated. Earlier trials indicated this procedure effectively eliminated microarthropods and nematodes. Microcosms were then inoculated with 2 ml of a suspension of bacteria and fungi prepared by homogenizing 15 g of field-moist forest litter in 150 ml deionized water and filtering the homogenate through a 5 μm Nucleopore filter. Dilution plating indicated this inoculum contained 1.3×10^7 bacteria and 5.4×10^4 fungal propagules. The inoculum may also have contained some small amoebae and flagellates (Frey *et al.*, 1985). No attempt was made to quantify protozoan numbers in the inoculum or microcosms.

Following inoculation, microcosms were covered with cheesecloth to allow for gas exchange and maintained at room temperature (25°C) and at 30% moisture by daily additions of deionized water. Total respiration was measured every 2–3 days for 56 days using a flow-through respirometer which allowed for simultaneous measurements in 20 microcosms and 4 blanks. Carbon dioxide-free air flowed through each container at a rate of 18.5 ml s^{-1} and into a CO_2 trap containing 50 ml of 0.01 N NaOH. Carbon dioxide was collected for 4 h and total CO_2 evolved was determined by titration with 0.02 N HCl, following addition of 1 ml of 1 N BaCl.

Respiration rates were allowed to stabilize for 2 weeks and on day 14 the microcosms were divided into two groups of 10 each so that pre-treatment means and variances between control and treatment groups were similar. The treatment then received 132 mg of naphthalene per container, equivalent to typical field application rates of 100 g m^{-2}. The other group served as an unmanipulated control. The cheesecloth covering allowed for volatilization of naphthalene and two additional naphthalene applications were made on days 28 and 42.

After 56 days respiration measurements were terminated and microcosms were destructively sampled. Litter and soil from 4 control and 4 naphthalene-treated microcosms was extracted on Tullgren funnels to check for accidental reintroduction of microarthropods. None was found. The litter and soil was oven-dried (50°C), weighed and ground and subsamples were analyzed for total Kjeldahl N. Soil from a second set of 4 control and 4 naphthalene-treated microcosms was extracted on Baermann funnels to check for reintroduction of nematodes. None was found. Litter from these microcosms was used to quantify numbers of bacteria and lengths of fungal hyphae. Litter from individual microcosms (0.3 g dry wt) was homogenized in 60 ml sterile water in a Virtis blender and appropriate dilutions were made. Bacteria were enumerated by epifluorescence microscopy following staining with fluorescein isothiocyanate (Babiuk and Paul, 1970). Lengths of total fungal hyphae were quantified using phase microscopy (Jones and Mollison, 1948) and FDA-active fungal hyphae by epifluoresence microscopy following staining with fluorescein diacetate (Soderstrom, 1977; Ingham and Klein 1982, 1984). Soil from a third set of 4 control and 4 naphthalene-treated microcosms was extracted with 2 N KCl and concentrations of extractable NH_4-N and $NO_3^- + NO_2$-N were determined on a Tecator autoanalyzer. Subsamples of these soils were used to determine numbers of soil bacteria and total and FDA-active fungi using the methods outlined above for litter.

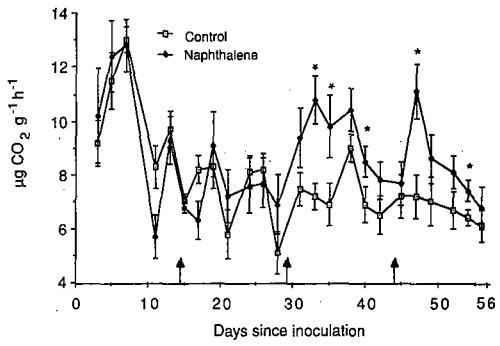

Fig. 1. Microbial respiration rates, expressed as μg CO_2 evolved g^{-1} of litter and soil h^{-1}, in litter–soil microcosms treated with naphthalene and untreated controls. Arrows indicate naphthalene applications. Asterisks indicate significant differences ($P < 0.05$) between treatments based on a one-tailed t-test of the null hypothesis that respiration rates in naphthalene-treated microcosms were not significantly higher than in controls.

RESULTS

Respiration rates in the microcosms increased for the first week following inoculation and then decreased to a level where they remained for most of study (Fig. 1). This initial flush of microbial activity was probably a response to increased amounts of available nitrogen following the microwave treatment

Table 1. Effects of naphthalene on numbers of bacteria g^{-1} of litter or soil and total and FDA-active fungi (m fungal hyphae g^{-1} of litter or soil) in soil-litter microcosms after 56 days. Values are expressed as $\bar{x} \pm$SE

	Control	Naphthalene	
Litter			
Bacteria (10^9 g^{-1})	4.5 ± 0.5	6.7 ± 0.7	$P < 0.05$[a]
Total fungi (m g^{-1})	470 ± 34	233 ± 18	$P < 0.001$
FDA-active fungi (m g^{-1})	13.5 ± 3.8	11.1 ± 2.8	NS
Soil			
Bacteria (10^9 g^{-1})	2.3 ± 0.2	4.1 ± 0.4	$P < 0.001$
Total fungi (m g^{-1})	28.4 ± 3.7	22.7 ± 2.8	NS
FDA-active fungi (m g^{-1})	2.4 ± 0.3	0.9 ± 0.2	$P < 0.01$

[a]Probability values based on a two-tailed t-test of the null hypothesis that means of control and naphthalene-treated microcosms were not different.

Table 2. Effects of naphthalene on litter mass, litter and soil Kjeldahl nitrogen and soil KCl-extractable NH_4-N and $NO_3^- + NO_2$-N pools in soil-litter microcosms after 56 days. Values are expressed as $\bar{x} \pm SE$

	Control	Naphthalene	
Litter			
Litter mass (g)	0.30 ± 0.00	0.30 ± 0.00	NS
Nitrogen (mg g^{-1})	10.2 ± 0.1	9.0 ± 0.1	$P < 0.001$[a]
Soil			
Nitrogen ($\mu g\, g^{-1}$)	943 ± 74	930 ± 8	NS
NH_4-N ($\mu g\, g^{-1}$)	6.73 ± 1.59	1.43 ± 0.11	$P < 0.005$
$NO_3^- + NO_2$-N ($\mu g\, g^{-1}$)	4.96 ± 1.51	0.24 ± 0.12	$P < 0.05$

[a]Probability values based on a two-tailed t-test of the null hypothesis that means of control and naphthalene-treated microcosms were not different.

(Speir *et al.*, 1986). There was no significant effect of naphthalene on total respiration following the first application on day 14. However, total respiration did increase significantly following the second and third naphthalene applications on days 28 and 42 (Fig. 1). In both cases the maximum increase occurred 3 days following the naphthalene additions.

Lengths of total fungal hyphae g^{-1} of litter at the end of the study were significantly lower in the naphthalene treatment (Table 1). FDA-active fungal hyphae in the litter were also slightly lower in the naphthalene treatment, but the difference was not significant. Lengths of total fungal hyphae in the soil of naphthalene-treated and control microcosms were not significantly different, but lengths of FDA-active fungal hyphae were significantly lower in the naphthalene treatment (Table 1). Numbers of bacteria g^{-1} of litter or soil were significantly higher in both litter and soil of naphthalene-treated microcosms (Table 1).

There was no difference in litter mass loss between treatments. In both treatments 0.30 g of litter, or 89% of the initial amount, remained at the end of the study (Table 2). In both treatments the concentration of nitrogen in the litter increased significantly by the end of the study. Nitrogen concentrations increased from 7.74 mg g^{-1} initially to 10.19 and 9.02 mg g^{-1} in the control and naphthalene treatments, respectively (Table 2). This 12% difference in final N concentrations was statistically significant ($P < 0.001$).

Final concentrations of total Kjeldahl N in the soil of control and naphthalene-treated microcosms were not significantly different from one another (Table 2) or from the initial concentration. However, there were significant differences in the amount of KCl-extractable N between treatments. There was approximately five times more extractable NH_4-N and 21 times more extractable $NO_3^- + NO_2$-N in control soils than in soils from the naphthalene-treated microcosms (Table 2).

Total amounts of nitrogen in litter, soil and whole microcosms were calculated from mass and N concentration data. Final amounts of litter N in the two treatments were significantly different from each other and from the initial amount of litter N (Table 3). Absolute amounts of litter N increased by about 4% in the control microcosms (net immobilization), while absolute amounts of litter N in the naphthalene treatment decreased by about 6% (net mineralization) (Table 3). Total amounts of N in the soil were more variable and differences between treatments were not significant.

Table 3. Total Kjeldahl nitrogen in litter and soil of microcosms treated with naphthalene and untreated controls. Nitrogen content is based on analysis of four individual microcosms from each treatment after 56 days. Values are reported as $\bar{x} \pm SE$. Analysis of variance indicated significant treatment effects on litter N only

	Initial	Control	Naphthalene
Litter (mg N)	2.94 ± 0.02^a	3.03 ± 0.03^b	2.73 ± 0.03^c
Soil (mg N)	17.66 ± 1.24	18.11 ± 1.42	17.86 ± 0.16
Total (mg N)	20.60 ± 1.23	21.13 ± 1.41	20.59 ± 0.15

Letters indicate significant differences ($P < 0.001$) based on Tukey's honestly significant difference test following ANOVA.

DISCUSSION

A major criticism of the use of biocides to exclude groups of organisms in field studies is their lack of specificity and potential for direct effects on non-target organisms. Biocides may affect organisms other than the target group in two ways, only one of which is necessarily undesirable. Reducing or eliminating the target group of organisms may secondarily affect other groups by altering interactions among these groups. For example, reducing microarthropods should subsequently affect the microbial community if microarthropod–microbial interactions significantly influence microbial activity or abundances. These secondary or "indirect effects" are expected and, in fact, provide insight into how the target group interacts with other organisms. However, biocides may also have undesirable direct "non-target effects" on organisms other than the target group. In this case the biocide directly affects non-target organisms, making it difficult, or impossible, to separate the effects of reducing the target group from the effects of the biocide itself. In spite of these problems, biocides have been widely used, partly because of the lack of viable alternative methods to study the role of soil organisms in the field.

Naphthalene has generally been regarded as having limited non-target effects, relative to other biocides used to reduce soil arthropods (i.e. Seastedt and Crossley, 1983). However, our results indicate naphthalene directly affects microbial activity and abundances in small soil-litter microcosms. Total microbial respiration increased with the second and third applications of naphthalene (Fig. 1). The lack of response to the first application may be attributed to the need to select for a microbial population capable of utilizing naphthalene as a carbon source. Studies of naphthalene degradation in water and sediment microcosms have indicated that the rate of degradation is related to population levels of hydrocarbon-

degrading microbes (Heitkamp *et al.*, 1987). The increase in respiration rates was probably due to increased bacterial activity in the naphthalene treatment. Fungi are apparently unable to use naphthalene as a sole carbon source (Heitkamp *et al.*, 1987) and our results indicate a reduction of total and FDA-active fungi in litter and soil, respectively, treated with naphthalene (Table 1). These results are in agreement with Newell *et al.* (1987), who found that naphthalene reduced the radial growth of fungal cultures in laboratory containers, and suggest that naphthalene has a detrimental effect on fungi.

Naphthalene altered nitrogen dynamics in the litter and soil of these microcosms. It slowed the increase in N concentration in the litter (Table 2) and resulted in net mineralization of N, as opposed to net immobilization in the controls (Table 3). Although these differences were small (10%), they were statistically significant and may be expected to increase during time as decomposition proceeds. Differences in litter N dynamics may be due to reduced fungal densities which could have resulted in less retention of litter N during decomposition or less translocation of N from soil to litter. Naphthalene also reduced the amount of extractable NH_4-N in the soils by a factor of 5 and the amount of extractable $N_3^- + NO_2$-N by a factor of 20. A likely explanation is that the greater numbers of soil bacteria in the naphthalene treatment (Table 1) increased microbial immobilization of N in the soil.

These results were obtained in simplified microcosms under laboratory condition. The microcosms used did contain a mix of bacteria and fungi obtained from field sources, received naphthalene at field application rates, and were open to gas exchange to prevent excessive build-up of naphthalene vapors. However, the effects of naphthalene in small enclosures may differ from those in field applications. For example, data from two recent field studies in a forest and agricultural system did not indicate a reduction in litter fungi in naphthalene-treated plots, relative to untreated controls (J. M. Blair *et al.*, unpublished data; P. F. Hendrix *et al.*, unpublished data). These studies also did not note any reduction in N retention in the litter during decomposition. Still, it is not possible to separate indirect and non-target effects in the field.

Without suitable alternative methods to manipulate microarthropods in the field, naphthalene and other biocides will continue to be used in field studies. We suggest that pre-experimental field trials be performed to determine minimum effective dosages in the system to be studied. It may be possible to use lower doses and less frequent application rates of naphthalene and still maintain effective microarthropod reduction, which would tend to minimize any non-target effects. However, assuming that the non-target effects observed in this study may occur in the field as well, caution should be used in ascribing any changes in soil processes in naphthalene-treated plots solely to reduction in microarthropod numbers.

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