APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Oct. 1995, p. 3537–3543 Vol. 61, No. 10
0099-2240/95/$04.00+0


# Role of Pore Size Location in Determining Bacterial Activity during Predation by Protozoa in Soil

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Received 15 February 1995/Accepted 13 July 1995

**The predation of a luminescence-marked strain of *Pseudomonas fluorescens* by the soil ciliate *Colpoda steinii* was studied in soil microcosms. Bacterial cells were introduced in either small (neck diameter, <6 μm) or intermediate-sized (neck diameter, 6 to 30 μm) pores in the soil by inoculation at appropriate matric potentials, and ciliates were introduced into large pores (neck diameter, 30 to 60 μm). Viable cell concentrations of bacteria introduced into intermediate-sized pores decreased at a greater rate than those in small pores, with reductions in bacterial populations being accompanied by an increase in viable cell numbers of the ciliate. The data indicate that the location of bacteria in small pores provides significant protection from predation. In the absence of *C. steinii*, the level of metabolic activity of the bacterial population, measured by luminometry, decreased at a greater rate than cell number, and the level of luminescence cell$^{-1}$ consequently decreased. The decrease in levels of luminescence indicates a loss of activity due to starvation. During predation by *C. steinii*, the level of the activity of cells introduced into small pores fell in a similar manner. The level of cell activity was, however, significantly greater for cells introduced into intermediate-sized pores, despite their greater susceptibility to predation. The data suggest that increased activity arises from a release of nutrients by the predator and the greater accessibility of bacteria to nutrients in larger pores. Nutrient amendment of microcosms resulted in increases in bacterial populations to sustained, higher levels, while levels of luminescence increased transiently. The predation of cells introduced into intermediate-sized pores was greater, and there was also evidence that the level of activity of surviving bacteria was greater for bacteria in intermediate-sized but not small pores.**

Protozoan grazing is believed to be a significant factor controlling the abundance of bacteria in soil and in determining the establishment of bacterial inocula (1, 4, 12, 23). The efficiency of protozoan predation depends to a large extent on free water within the soil, and grazing is effectively restricted to moist conditions in which pores in the soil of sufficient size are filled with water (26). Soil water content and matric potential will also determine the pore size location of bacterial inocula, which, in turn, will affect the accessibility of bacteria to protozoal predators (2). The location of bacteria in pores with neck sizes of 3 to 6 μm will reduce predation by excluding protozoa (10, 13). Heijnen et al. (14) demonstrated the greater survival of bacterial inocula introduced into pores with neck sizes of less than 6 μm. Wright et al. (29) also found an improved survival of *Pseudomonas fluorescens* located in a class of similar pore size in nonsterile soil inoculated with *Colpoda steinii*. In addition to protective effects, the location of bacteria in small pores may affect their ability to utilize nutrients released during predation. For example, Killham et al. (17) found lower rates of carbon turnover when organic substrates were located in smaller pores and at a lower matric potential, conditions under which the diffusion of nutrients will be reduced.

The reduction in bacterial numbers during predation is not necessarily accompanied by a reduction in the level of activity of the surviving bacterial population. Gude (11) suggested that predation led to a net regeneration of nutrients, which are immobilized in bacterial cells, and there is evidence that ammonium or organic N (15, 18) and phosphorus (5) are released during predation by protozoa, thereby increasing the nutrient status of the soil. Bacterial activity during predation has been estimated by the measurement of respiration rates (6) and the production of ammonium (18), but such techniques do not distinguish between the activities of predator and prey. The measurement of nitrogen fixation provides such a distinction (8) but determines only the level of activity of a small component of the bacterial community. Luminescence-based marker systems provide a means for selective quantification of the activity of soil bacterial inocula. Such systems involve the introduction of the structural genes for luciferase (*luxAB*), originally isolated from naturally luminescent marine organisms (e.g., *Vibrio harveyi*). The metabolic activity of marked cells is determined by the measurement of luminescence and by luminometry and correlates with other measures of metabolic activity (20, 21). Luminometry is much more sensitive, rapid, and accurate than conventional techniques for measurement of activity and, importantly, measures the activity of the marked organism only. It is therefore ideal for studies of the effects of predation on the activity of a specific bacterial inoculum. In this study, the bacterial prey was a strain of *P. fluorescens* chromosomally marked with *luxAB* genes, and the predator was the soil ciliate protozoan *C. steinii*. Cells were located in pores of required size by introducing them at particular matric potentials, with reference to the soil-moisture-release characteristic.

The aims of the study were (i) to determine whether the location of *P. fluorescens* in small-pore-size classes could pro-

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tect cells from predation by *C. steinii*, (ii) to determine whether the turnover of nutrients following predation stimulated bacterial activity, and (iii) to assess the effects of location in pores of different sizes on bacterial activity and predation following nutrient amendment. *C. steinii* was chosen to test this hypothesis because of its relatively large size (20 to 30 μm) and because other protozoal predators, in particular amoebae, can gain access to small pores by deformation of shape.

## MATERIALS AND METHODS

**Bacterial strains, plasmids, and cultural conditions.** Predation was studied between a bacterial prey, *P. fluorescens* 10586s/FAC510, and a bacteriophagous, predatory ciliate, *C. steinii. P. fluorescens* 10586s/FAC510 is chromosomally marked with the luciferase structural genes, *luxA* and *-B*, isolated from *V. harveyi* and under control of the tetracycline resistance gene promoter (3). The parent strain was obtained from the National Collections of Industrial and Marine Bacteria. Luciferase production by the luminescence-marked strain is constitutive, and the absence of functional *luxCDE* genes necessitates the exogenous addition of the aldehyde substrate for light output. This strain is also resistant to kanamycin and ampicillin. Routine culture of *P. fluorescens* 10586s/FAC510 was carried out at 25°C in Luria-Bertani broth (tryptone, 10 g; NaCl, 5 g; yeast extract, 5 g; glucose, 1 g; distilled water, 1 liter [pH 7]) containing 20 μg of kanamycin per ml, and luminescence characteristics during batch growth are described by Amin-Hanjani et al. (3).

*C. steinii* was originally isolated from soil and was kindly provided by J. F. Darbyshire (Macaulay Land Use Research Institute, Aberdeen, Scotland). The ciliate was routinely cultured on *P. fluorescens* 10586 (the nonluminescent, parent strain) in the medium of Prescott and James (see reference 22) by the procedure described by Wright et al. (29).

**Soil microcosms.** Predation was studied in a sandy loam soil belonging to the Insch series (cation exchange capacity, 9.31 cmol $kg^{-1}$; organic matter, 3.75%). The pH was adjusted to 7 by amendment with $Ca(OH)_2$, and soil was sieved (to <3 mm). Matric potentials required to locate inocula in particular pore-size classes were determined by calculating the effective neck diameter of the largest water-filled pore (in micrometers) with the equation diameter = 300/−matric potential (in kilopascals) (19). Volumetric water contents required to achieve the desired matric potentials were derived with reference to the moisture release characteristics of the soil (27), which were determined for autoclaved soil with the same bulk packing density used in microcosms. Soil was sterilized by autoclaving for 1 h at 121°C on three separate days.

Microcosms consisted of 9.6 g of sterile soil placed in sterile glass petri dishes (50 mm in diameter). Soil formed a 6-mm-deep layer equivalent to the height of two aggregates, and the soil therefore had no macrostructure. Bacterial inocula were prepared by harvesting exponentially growing cells of *P. fluorescens* 10586s/FAC510 (grown as described above) by centrifugation (8,800 × *g*, 4°C, 30 min). Cells were washed twice in 15 mM phosphate buffer and resuspended in 20 ml of buffer. Washed bacterial and ciliate cell suspensions were incubated overnight at 20°C before the inoculation of microcosms.

Bacteria were introduced into pores with neck diameters of <6 μm or with diameters in the range of 6 to 30 μm, while either sterile water or the ciliate was introduced into pores with neck diameters in the range of 30 to 60 μm. For the former situation, the bacterial suspension was diluted in a volume of sterile water, to give a matric potential of −50 kPa and a final cell concentration of $1 \times 10^8$ to $5 \times 10^8$ cells g of $soil^{-1}$ following an even distribution of 10-μl volumes over the soil surface. This ensured that the inoculum came into contact with each aggregate, with a subsequent equilibration within aggregates. After equilibration for 24 h, sufficient sterile water was added over a 6-h period to raise the potential to −10 kPa, filling pores with diameters in the range of 6 to 30 μm. The matric potential was then raised to −5 kPa by the addition of either sterile water or a suspension of *C. steinii*, adjusted to give a final cell concentration in the range of $10^3$ to $10^4$ cells $g^{-1}$. For the second situation, the procedure was the same, except that the matric potential was raised to −50 kPa by the addition of sterile water. Bacteria were then added to raise the matric potential to −10 kPa. In experiments involving nutrient amendment, sufficient complex growth medium was added 5 days after inoculation to increase the matric potential from −5 to −1 kPa, filling pores with neck diameters in the range of 60 to 300 μm. The growth medium was double-strength 523 medium (16) amended with 10 mg of sodium citrate per ml. Single-strength 523 medium consists of sucrose, 10 g; Casamino Acids, 8 g; $K_2HPO_4 \cdot 3H_2O$, 3.9 g; $MgSO_4 \cdot 7H_2O$, 0.3 g; yeast extract, 4 g; and distilled water, 1 liter.

Following inoculation, microcosms were incubated at 25°C in polystyrene boxes (50 by 50 by 50 cm), to minimize moisture loss through evaporation. At appropriate periods after inoculation with *C. steinii*, triplicate microcosms were destructively sampled for the determination of bacterial and ciliate viable cell concentrations and the measurement of luminescence.

**Enumeration of bacteria and protozoa.** Cells were extracted from soil by shaking 1 g of soil in 1 ml of 15 mM phosphate for 10 min with a Stuart wrist-action shaker to form a homogeneous suspension. Viable cell counts of *P. fluorescens* 10586s/FAC510 were obtained by the construction of a decimal dilution series of the resultant suspension in 15 mM phosphate buffer and by spread plating 100-μl samples on triplicate plates containing Luria-Bertani broth, which was supplemented with 20 μg of kanamycin per ml and solidified by the addition of 1.5% (wt/vol) technical no. 3 agar (Oxoid). Colonies were counted after the plates were incubated for 48 h at 30°C; the lower detection level was approximately 100 cells $ml^{-1}$.

The *C. steinii* viable cell concentration was determined by the microtiter plate most probable number method of Darbyshire et al. (9). A soil suspension was obtained as described above, and 25-μl samples were placed into a row of eight wells in a microtiter plate, each containing 100 μl of a culture of *P. fluorescens* 10586 in Prescott and James medium. A series of fivefold dilutions was constructed by successive transfer into further rows of wells, each containing similar volumes of *P. fluorescens* cultures, to a final dilution of $5^{-11}$. Microtiter plates were sealed with parafilm and incubated in the dark at 20°C. The presence of *C. steinii* cells in individual wells was determined with an inverted microscope after incubation for 7 and 14 days.

Bacterial and ciliate counts are expressed as cells gram of oven-dry-weight $soil^{-1}$.

**Luminescence measurements.** Luminescence was measured with an LKB 1251 luminometer with continuous mixing. Samples (1 ml) of soil suspensions (prepared as described above) were mixed with 1 μl of *n*-decyl aldehyde for 10 s and incubated for 4 min, as described by Amin-Hanjani et al. (3). Light output was then measured by integration over a period of 10 s, and values are expressed as relative light units (RLU) gram of oven-dry-weight $soil^{-1}$, 1 RLU being equivalent to 1 mV 10 $s^{-1}$.

**Statistical analysis.** All microcosm experiments were carried out in triplicate. Treatments were compared by two-way analysis of variance of $\log_{10}$-transformed values of viable cell concentrations, levels of luminescence, and levels of luminescence $cell^{-1}$, and differences were assessed during the total period over which a treatment was applied. Variability is indicated in the figures as minimum significant difference values at the 0.05 level of significance, calculated from analysis of variance.

## RESULTS

In the first series of experiments, predation by *C. steinii* was investigated after the introduction of *P. fluorescens* into either small or intermediate-sized pores in soil microcosms. Changes in bacterial and ciliate concentrations, and bacterial luminescence, were determined for a period of 270 h. In the second series of experiments, the effect of a nutrient flush on predation and bacterial activity was investigated by nutrient amendment of soil microcosms 150 h after the inoculation of bacteria into either small or intermediate-sized pores.

**Influence of location in pores of various sizes on predation and bacterial activity.** In the absence of *C. steinii*, the concentration of *P. fluorescens* cells introduced into small pores (neck diameter, <6 μm) decreased from an initial concentration of $2.2 \times 10^8$ to $7.2 \times 10^7$ cells g of $soil^{-1}$ after 270 h (Fig. 1a). *C. steinii* was introduced into large pores (neck diameter, 30 to 60 μm) and separated from bacteria by a buffer zone of sterile water in intermediate-sized pores (neck diameter, 6 to 30 μm). This resulted in a decrease in numbers of *P. fluorescens* to $1.7 \times 10^7$ cells g of $soil^{-1}$ within 102 h (Fig. 1a). The decrease in *P. fluorescens* numbers was significantly ($P \leq 0.001$) greater than that in the absence of the ciliate. Decreases in levels of luminescence paralleled changes in viable cell concentration (Fig. 1b), with a significantly greater decrease in luminescence levels in the presence of *C. steinii*. The level of luminescence per viable cell decreased slightly with time but was not affected by the presence of *C. steinii* ($P = 0.919$) (Fig. 1c). Values ranged from $5.0 \times 10^{-6}$ to $2.8 \times 10^{-6}$ RLU $cell^{-1}$ and $1.2 \times 10^{-5}$ to $3.2 \times 10^{-6}$ RLU $cell^{-1}$ in the absence of and presence of *C. steinii*, respectively. Thus, the decrease in the bacterial population in the presence of *P. fluorescens*, introduced into small pores, did not affect cellular metabolic activity, which was measured as luminescence $cell^{-1}$.

A decrease in the *P. fluorescens* cell concentration in the presence of *C. steinii* was accompanied by an increase in the ciliate population (Fig. 2), confirming the decrease to be due to predation. Bacterial and ciliate cell concentrations were used to estimate protozoal yield on bacterial cells and the doubling

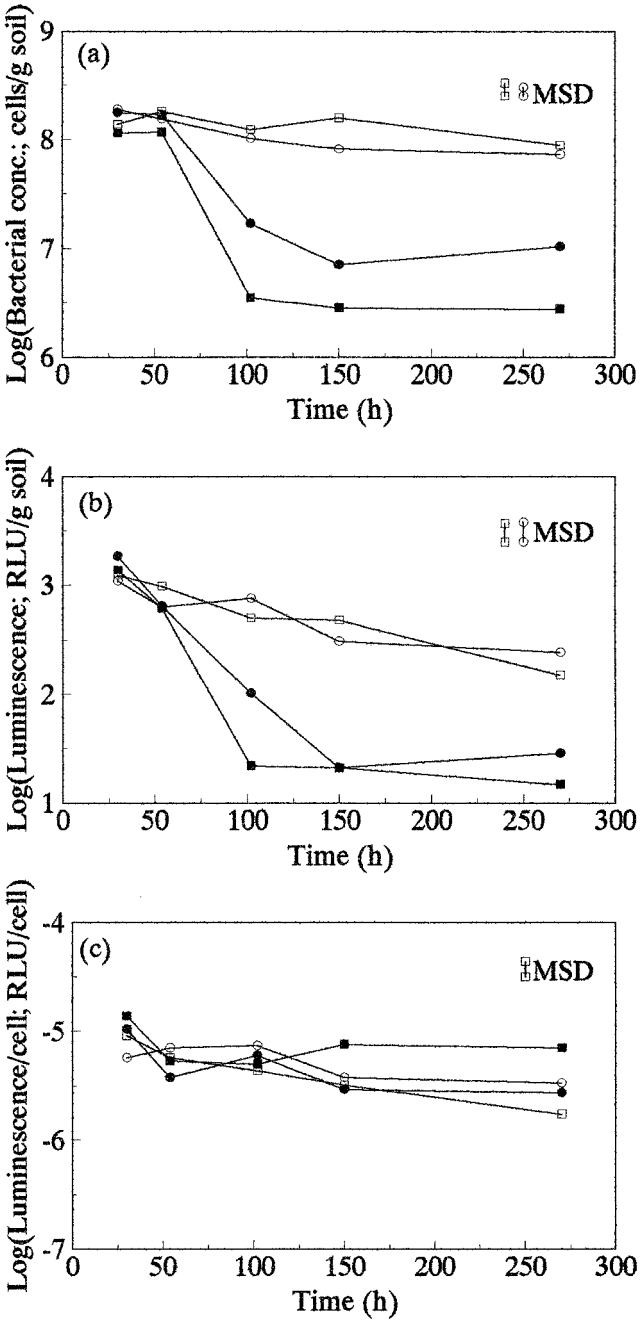


FIG. 1. Changes in (a) viable cell concentrations, (b) luminescence, and (c) luminescence viable cell$^{-1}$ following the introduction of *P. fluorescens* into small pores (neck diameter, <6 μm) (○, ●) or intermediate-sized pores (neck diameter, 6 to 30 μm) (□, ■) and the introduction of sterile water (○, □) or *C. steinii* (●, ■) into large pores (neck diameter, 30 to 60 μm). Individual points are the $\log_{10}$-transformed means of triplicate samples. Error bars are minimum significant difference (MSD) values calculated from a two-way analysis of variance for $P \leq 0.05$. Bars are omitted where treatments were not significantly different ($P > 0.05$).

time for *C. steinii* (Table 1). Yield was calculated as increase in ciliate population/decrease in bacterial population.

The ciliate doubling time was calculated from a semilogarithmic plot of ciliate cell concentration versus time. As major changes in bacterial and ciliate cell concentrations occurred between two sampling points (54 and 102 h), calculated doubling times represent maximum values.

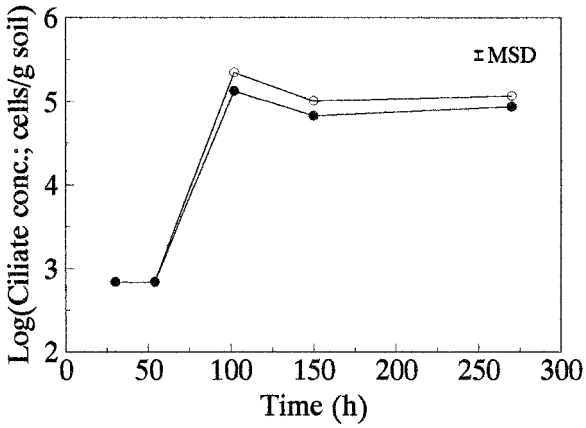


FIG. 2. Changes in viable cell concentrations of *C. steinii* consuming *P. fluorescens* located in small (○) or large (●) pores. Data are analyzed and presented as described in the legend for Fig. 1.

The introduction of bacterial cells into intermediate-sized pores did not significantly affect changes in viable cell concentration ($P = 0.093$) or luminescence ($P = 0.674$) of *P. fluorescens* in the absence of *C. steinii* (Fig. 1a and b). The level of luminescence decreased at a slightly faster rate than viable cell concentration, and the level of luminescence cell$^{-1}$ consequently showed a significant decrease during the course of the experiment (Fig. 1c). In the presence of *C. steinii*, introduced into large pores, bacterial numbers decreased by approximately 2 orders of magnitude, from $1.2 \times 10^8$ cells g of soil$^{-1}$ to $3.6 \times 10^6$ cells g of soil$^{-1}$ at 102 h. Changes in viable cell concentration were similar to changes in luminescence, and both were significantly greater than those in the absence of *C. steinii* ($P < 0.001$). Reductions in viable cell concentration and the level of luminescence were also significantly greater than those when bacterial cells were introduced into small pores ($P < 0.001$). Cell activity, measured as luminescence cell$^{-1}$, decreased initially from $1.4 \times 10^{-5}$ to $5.1 \times 10^{-6}$ RLU cell$^{-1}$ but subsequently increased significantly ($P = 0.026$), reaching $7.1 \times 10^{-6}$ RLU cell$^{-1}$ by 270 h. Luminescence cell$^{-1}$ values were significantly greater for bacterial cells introduced into large pores following predation ($P \leq 0.001$).

Ciliate numbers increased with the decrease in the bacterial population (Fig. 2), and growth parameters are given in Table 1. A reliable comparison of the values of doubling times is difficult because of the lack of data during changes in populations. Protozoal yield, measured as protozoa produced per

TABLE 1. Estimated values for yield and doubling time for *C. steinii* during the predation of *P. fluorescens* 10586s/FAC510 located in different soil pore-size classes

| Expt | Pore size (μm) | Yield (ciliate bacterium$^{-1}$)[a] | Doubling time (h) |
|---|---|---|---|
| 1 | <6 | $2.2 \times 10^{-4}$ | 5.8 |
| | 6–30 | $1.1 \times 10^{-4}$ | 6.3 |
| 2 prior to nutrient amendment | <6 | $9.7 \times 10^{-4}$ | 12.1 |
| | 6–30 | $5.8 \times 10^{-4}$ | 11.1 |
| 2 after nutrient amendment | <6 | ND | 10.3 |
| | 6–30 | ND | 25.6 |

[a] ND, not determined. It was not possible to measure yield following nutrient amendment because of increases in bacterial numbers.

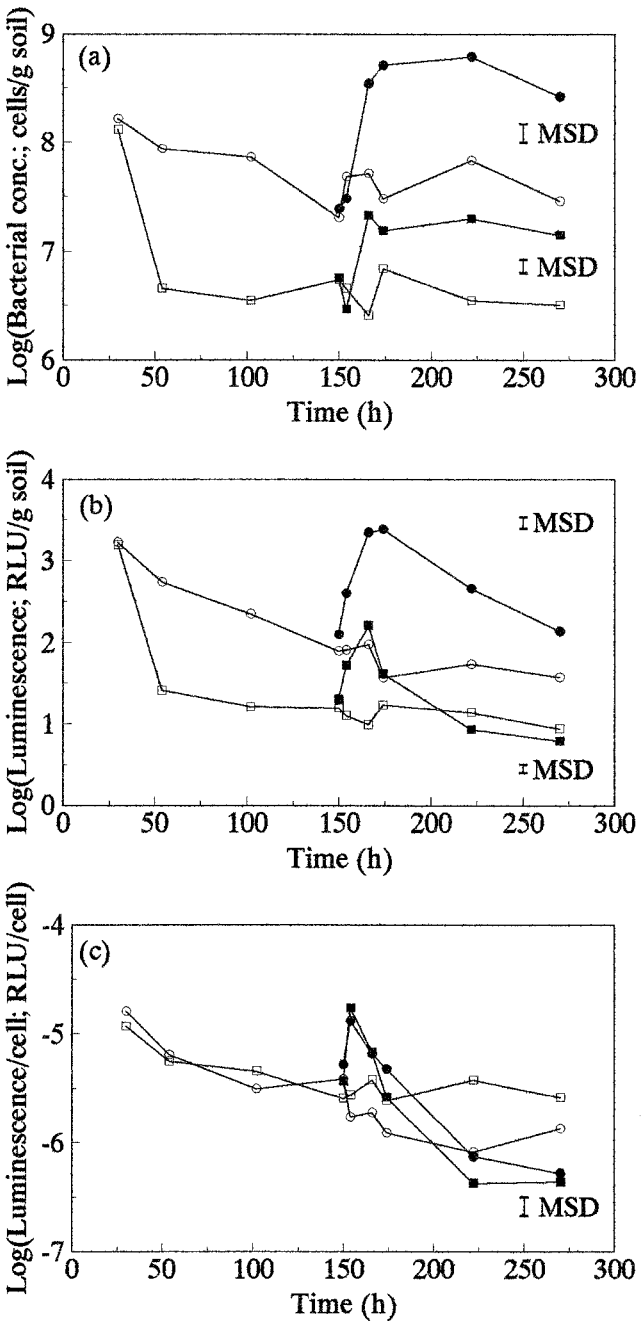


FIG. 3. Changes in (a) viable cell concentrations, (b) luminescence, and (c) luminescence viable cell$^{-1}$ following the introduction of *P. fluorescens* into small pores and the introduction of sterile water (○, ●) or *C. steinii* (□, ■) into large pores (neck diameter, 30 to 60 μm). Microcosms were amended, at 150 h, with either sterile water (○, □) or double-strength complex medium (●, ■). Data are presented as described in the legend for Fig. 1.

bacterial cell consumed, was, however, greater when bacteria were located in small pores.

**Influence of nutrient amendment on predation and bacterial activity.** Prior to nutrient amendment, changes in viable cell concentrations of bacteria and in luminescence (Fig. 3a and b) were similar to those described above when bacterial cells were introduced into small pores. The presence of *C. steinii* reduced bacterial populations and luminescence by approximately 1.5 orders of magnitude. Luminescence activity per cell decreased slightly with time but was unaffected by the presence of the ciliate. At 150 h, either nutrient (double-strength complex growth medium) or sterile water was added to microcosms inoculated with *P. fluorescens* or both *P. fluorescens* and *C. steinii*, filling pores of neck diameters of up to 300 μm. The addition of sterile water to microcosms containing only *P. fluorescens* led to a small increase in the viable cell concentration in the absence of *C. steinii*, which was maintained until 270 h (Fig. 3a). A much larger increase, greater than 1 order of magnitude, occurred within 24 h of nutrient amendment, and numbers were maintained at this higher level for the remainder of the experiment. A similar effect was seen in microcosms inoculated with both *P. fluorescens* and *C. steinii*, where the increase was rapid but less marked (Fig. 3a).

Amendment with sterile water had a smaller proportional effect on luminescence than on viable cell concentration, but nutrient amendment led to significantly greater increases in levels of luminescence, in the presence and absence of *C. steinii* (Fig. 3b). Increases were, however, transient and luminescence approached or fell to levels similar to that of unamended microcosms by 270 h. The relative effects on cell concentration and luminescence are reflected in luminescence cell$^{-1}$ values (Fig. 3c). After the addition of sterile distilled water, levels of luminescence cell$^{-1}$ continued to decline in the absence of *C. steinii*. Predation, however, led to significantly ($P \leq 0.001$) higher cell activities, which were maintained until the termination of the experiment. Nutrient amendment resulted in sharp increases in cell activity, but within 24 h, luminescence cell$^{-1}$ fell to initial levels and then fell below those in unamended microcosms.

Decreases in bacterial populations were associated with an increase in numbers of *C. steinii* both before and after nutrient addition. The addition of sterile water led to a slight increase in ciliate numbers, but a much greater increase, approximately 1 order of magnitude, was seen following nutrient amendment (Fig. 4). Values of yield and doubling time calculated prior to the nutrient amendment were greater than in the first experiment, and doubling times calculated initially and following amendment were similar (Table 1).

When bacterial cells were introduced into intermediate-sized pores, initial changes in viable cell concentrations and levels of luminescence and luminescence cell$^{-1}$ were similar to those described in the first experiment, although the decrease in the levels of luminescence cell$^{-1}$ with time was slightly

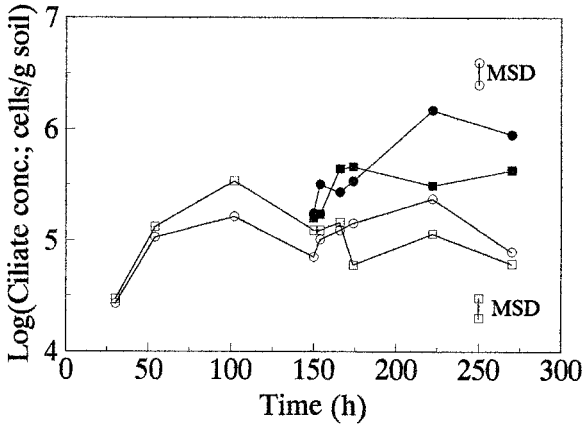


FIG. 4. Changes in viable cell concentrations of *C. steinii* consuming *P. fluorescens* located in small (○, ●) or large (□, ■) pores. Microcosms were amended, at 150 h, with either sterile water (○, □) or double-strength complex medium (●, ■). Data are analyzed and presented as described in the legend for Fig. 1.

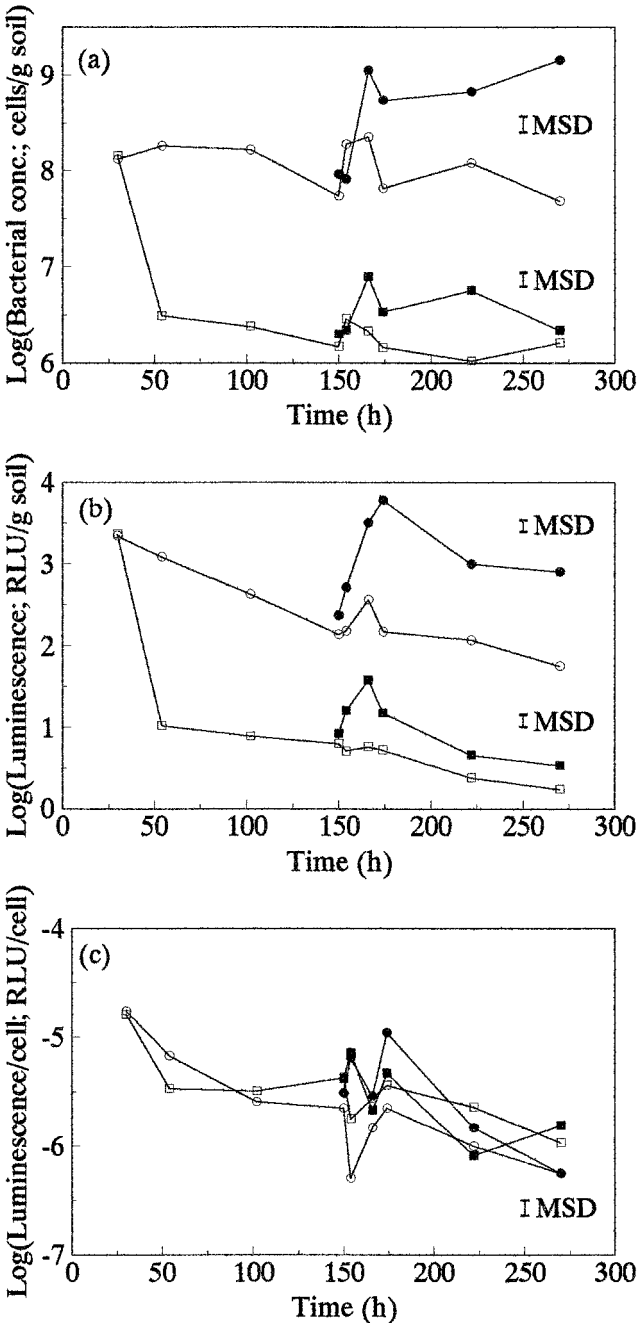


FIG. 5. Changes in (a) viable cell concentrations, (b) luminescence, and (c) luminescence viable cell$^{-1}$ following the introduction of *P. fluorescens* into intermediate-sized pores and the introduction of sterile water (○, ●) or *C. steinii* (□, ■) into large pores (neck diameter, 30 to 60 μm). Microcosms were amended, at 150 h, with either sterile water (○, □) or double-strength complex medium (●, ■). Data are presented as described in the legend for Fig. 1.

greater (Fig. 5). The addition of sterile water led to a slight increase in viable cell concentrations, in the presence and absence of *C. steinii*, and to a small burst in luminescence activity in the absence of the ciliate. Nutrient amendment led to an increase in viable cell concentration, which was greater in the absence of *C. steinii*. Increased cell concentrations were maintained until 270 h. Nutrient amendment led to transient increases in levels of luminescence for approximately 24 h, after which luminescence levels fell. The addition of complex nutrients led to a burst in cell activity which did not occur after the addition of sterile distilled water (Fig. 5c). Luminescence cell$^{-1}$ values for the two amendments then converged, although there was some indication of a further increase in bacterial activity measured at 270 h in microcosms inoculated with *C. steinii* and amended with nutrient. Nutrient amendment also gave rise to an increase in ciliate numbers, which corresponded to the decrease in the bacterial population (Fig. 4). The calculated doubling time was twice that following the nutrient amendment of cells located in small pores.

## DISCUSSION

An investigation of the role of spatial location in determining bacterial activity during predation requires reliable techniques for locating cells predominantly in particular pore-size classes. In our study, this was achieved by the adjustment of the antecedent matric potential and the subsequent uniform addition of a specific volume of inoculum with reference to the moisture-release-characteristic curve for the soils used. The use of calcofluor-stained cells and resin impregnation of inoculated soil enabled White et al. (27) to confirm the validity of this approach. Further indirect evidence of effective inoculum placement has been obtained by partial chloroform fumigation techniques (28).

The results obtained here confirm that the location of *P. fluorescens* in small pores provides protection from predation by *C. steinii*. Wright et al. (29) also found a reduced predation of *P. fluorescens* cells located in pores with a neck diameter of <6 μm, but their use of nonsterile soil did not eliminate the effects of indigenous soil protozoa. In this study, *C. steinii* was the sole predator, and significantly greater reductions in bacterial numbers were observed when cells were introduced into intermediate-sized pores which were accessible to the ciliate. The correlation between decreases in bacterial numbers and increases in ciliate populations confirms that the former were due to predation. Loss of bacterial cell viability will also have occurred, although microcosms containing only *P. fluorescens* indicated that this loss was small in relation to the loss due to predation. Size exclusion therefore increased survival of the bacterial prey, although the fact that predation occurred when bacteria were introduced into small pores indicates that the technique used for determining pore size location was not completely effective or that movement of cells occurred after inoculation. These results also confirm the findings of Heijnen et al. (14), who observed higher population densities of bacteria located in pores with a neck diameter of <6 μm compared with pores with a neck diameter of >6 μm.

The decrease in levels of luminescence during predation may have been due to decreased cell activity or a decrease in bacterial population size. Seale et al. (24) investigated the predation of the naturally luminescent bacterium *Xenorhabdus luminescens* by freshwater microflagellates and interpreted reductions in levels of luminescence to be directly related to reductions in cell numbers due to predation. In our study, the measurement of both cell concentration and luminescence provided an estimation of cell activity, on the assumption that the efficiency of cell recovery and viable cell concentration did not vary significantly in the different treatments. Cell activity was found to decrease when cells were introduced into soil, even in the absence of predation. Luminescence has been shown to be a measure of metabolic activity (20, 21), and the reductions in levels of luminescence cell$^{-1}$ are likely to have resulted from the reductions in substrate and nutrient supplies and the possible entry of cells into a dormant state.

The application of the luminescence-based marker system provides the first study of predation in which distinction between metabolic activity of the prey and the predator has been possible. Other attempts have been limited by the measurement of processes, such as respiration, which are shared by both predator and prey or by the measurement of processes, such as nitrogen fixation, which are restricted to a fraction of the bacterial community. Luminometry enables specific and rapid measurement of bacterial activity and showed predation to have little effect on the activity of bacteria introduced into small pores, from which the ciliate would be excluded. The lack of an effect on the activity of cells in small pores may be because predation did not result in a sufficient turnover of nutrients for the detection of changes in activity or because the products of the turnover of nutrients failed to diffuse into small pores and activate cells.

The increase in luminescence activity per cell during predation of bacteria introduced into large pores provides the first direct evidence for the stimulation of prey activity through predation and the turnover of nutrients immobilized in the prey, earlier suggested by the work of Cole et al. (5) and van Veen and Kuikman (25). Thus, although predation reduced the size of the bacterial population, the level of activity of the survivors was increased through growth on nutrients released by predation. This may result from the predation of a larger number of bacteria and the release of greater quantities of nutrients or from a greater access to nutrients following turnover. Changes in the luminescence activity of bacteria introduced into large pores indicate the latter case to be more likely. The predation of this population led to a significant increase in the level of luminescence $cell^{-1}$ and a reduction in bacterial numbers of 2 rather than 1 order of magnitude, as found for the predation of cells in small pores. Thus, only 10% more cells were consumed when cells were located in large pores. The turnover of these cells is unlikely to have given rise to the difference in levels of luminescence activity per cell observed, and it is more likely that the close proximity of bacterial and ciliate cells increased the availability of nutrients to bacteria. Cells in small pores were therefore denied contact with nutrients as well as predators. Other possible contributory factors are greater oxygen limitation in small pores, cryptic growth, and a lack of correlation between activity and luminescence. Previous work (20, 21), however, indicates that metabolic activity and luminescence are directly related in strains constitutively expressing *luxA* and *-B* genes.

The amendment of microcosms with water led to a relatively small increase in levels of bacterial activity and in bacterial numbers, presumably because of the disturbance and mobilization of nutrients. Amendment with nutrients led to a much greater increase in cell numbers and a burst of luminescence. This demonstrates a further advantage of the luminescence marker system as a sensitive indicator of bacterial ecology in the soil. The increase in numbers was maintained because of efficient survival of cells, but the increased activity was short-lived because of the rapid utilization of nutrients.

The presence of the predator reduced increases in bacterial numbers and activity following nutrient amendment, but changes in the level of activity per cell were less evident than those described above. While the addition of distilled water led to greater levels of activity for cells located in small pores in the presence of *C. steinii*, the presence of the predator did not lead to differences in levels of activity after the nutrient addition. When bacterial cells were located in intermediate-sized pores, the addition of nutrients led to a short increase in cell activity levels but the presence of the predator had little effect on cell activity.

Values of yields and doubling times of the predator were estimated, although the lack of samples during rapid changes in population sizes make these values approximations only. Nevertheless, the yield was greater, and doubling times shorter, when ciliates were spatially separated from bacteria. Curds and Bazin (7) found that, in continuous culture, ciliates provided with excess prey grazed rapidly and increased in size. As food became scarce, ciliates became smaller but more efficient, with higher growth rates. In our study, *C. steinii* would have a greater food source when grazing on bacteria in large rather than smaller pores, where a portion of the population would be inaccessible. In the former case, the ciliate will have higher consumption rates but lower growth rates and lower yields.

In conclusion, this study illustrates the role of protozoal predation in the indirect release of nutrients from bacterial cells, using luminescence-based marker systems to measure specifically the activity of the bacterial prey. Further, the results demonstrate the important roles of pore size and the location of cells within pores in both protecting bacterial prey from predation and in determining the influence of released nutrients on bacterial activity.

## ACKNOWLEDGMENT

David Wright acknowledges receipt of a Natural Environment Research Council Postgraduate Studentship.

## REFERENCES

1. **Acea, M. J., C. R. Moore, and M. Alexander.** 1988. Survival and growth of bacteria introduced into soil. Soil Biol. Biochem. **20:**509–515.
2. **Alexander, M.** 1981. Why microbial predators and parasites do not eliminate their prey and hosts. Annu. Rev. Microbiol. **35:**113–133.
3. **Amin-Hanjani, S., A. M. Meikle, L. A. Glover, J. I. Prosser, and K. Killham.** 1993. Plasmid and chromosomally encoded luminescence marker systems for detection of *Pseudomonas fluorescens* in soil. Mol. Ecol. **2:**47–54.
4. **Clarholm, M.** 1984. Heterotrophic free-living protozoa: neglected microorganisms with an important task in regulating bacterial populations, p. 321–326. *In* M. J. Klug and C. A. Reddy (ed.), Current perspectives in microbial ecology. American Society for Microbiology, Washington D.C.
5. **Cole, C. V., E. T. Elliot, H. W. Hunt, and D. C. Coleman.** 1978. Trophic interactions in the soil as they affect energy and nutrient dynamics. V. Phosphorous transformations. Microb. Ecol. **4:**381–387.
6. **Coleman, D. C., R. V. Anderson, C. V. Cole, E. T. Elliot, L. Woods, and M. K. Campion.** 1978. Trophic interactions in the soil as they affect energy and nutrient dynamics. IV. Flows of metabolic and biomass carbon. Microb. Ecol. **4:**373–380.
7. **Curds, C. R., and M. J. Bazin.** 1977. Protozoan predation in batch and continuous culture. Adv. Aquat. Microbiol. **1:**115–176.
8. **Darbyshire, J. F.** 1972. Nitrogen fixation by *Azotobacter chroococcum* in the presence of *Colpoda steinii*. I. The influence of temperature. Soil Biol. Biochem. **4:**359–369.
9. **Darbyshire, J. F., R. E. Wheatley, M. P. Greaves, and R. H. E. Inkson.** 1974. A rapid micromethod for estimating bacterial and protozoan populations in soil. Rev. Ecol. Biol. Sol **11:**465–475.
10. **Foster, R. C.** 1988. Microenvironments of soil microorganisms. Biol. Fertil. Soils **6:**189–203.
11. **Gude, H.** 1985. Influence of phagotrophic processes on the regeneration of nutrients in two-stage continuous culture systems. Microb. Ecol. **11:**193–204.
12. **Habte, M., and M. Alexander.** 1977. Further evidence for the regulation of bacterial populations in soil by protozoa. Arch. Microbiol. **113:**181–183.
13. **Hattori, T.** 1988. Soil aggregates as microhabitats of microorganisms. Rep. Inst. Agric. Res. Tohoku Univ. **37:**23–36.
14. **Heijnen, C. E., C. H. Hok-A Hin, and J. A. van Veen.** 1991. Protection of *Rhizobium* by bentonite clay against predation by flagellates in liquid cultures. FEMS Microbiol. Ecol. **85:**65–72.
15. **Hunt, H. W., C. V. Cole, D. A. Klein, E. T. Elliot, and D. C. Coleman.** 1977. A simulation model for the effect of predation on bacteria in continuous culture. Microb. Ecol. **3:**259–278.
16. **Kado, C. I., M. G. Hesketh, and R. A. Langley.** 1972. Studies of *Agrobacterium tumefaciens*: characterisation of strains ID135 and B6 and analysis of the bacterial chromosome transfer RNA and ribosomes for tumour inducing ability. Physiol. Plant Pathol. **2:**47–57.
17. **Killham, K., M. Amato, and J. N. Ladd.** 1992. Effect of substrate location in soil and soil pore water regime on carbon turnover. Soil Biol. Biochem. **25:**57–62.

18. **Kuikman, P. J., J. D. van Elsas, A. G. Jansen, S. L. G. E. Burgers, and J. A. van Veen.** 1990. Population dynamics and activity of bacteria and protozoa in relation to their spatial distribution in soil. Soil Biol. Biochem. **22:**1063–1073.
19. **Marshall, K. C., and J. W. Holmes.** 1980. Soil physics, 2nd ed. Cambridge University Press, Cambridge.
20. **Meikle, A., L. A. Glover, K. Killham, and J. I. Prosser.** 1994. Potential luminescence as an indicator of activation of genetically modified *Pseudomonas fluorescens* in liquid culture and in soil. Soil Biol. Biochem. **26:**747–755.
21. **Meikle, A., K. Killham, J. I. Prosser, and L. A. Glover.** 1992. Luminometric measurement of population activity of genetically modified *Pseudomonas fluorescens* in the soil. FEMS Microbiol. Lett. **99:**217–220.
22. **Page, F. C.** 1976. An illustrated key to freshwater and soil amoebae, with notes on cultivation and ecology. Scientific Publication no. 34. Freshwater Biological Association, Ambleside, Cumbria, United Kingdom.
23. **Recorbet, G., C. Steinberg, and G. Faurie.** 1992. Survival in soil of genetically engineered *Escherichia coli* as related to inoculum density, predation and competition. FEMS Microbiol. Ecol. **101:**251–260.
24. **Seale, D. B., M. E. Voraas, D. Holen, and K. H. Nealson.** 1990. Use of bioluminescent bacteria, *Xenorhabdus luminescens*, to measure predation on bacteria by freshwater microflagellates. FEMS Micriobiol. Ecol. **73:**31–40.
25. **van Veen, J. A., and P. J. Kuikman.** 1990. Soil structural aspects of decomposition of organic matter by microorganisms. Biogeochemistry (Dordr.) **11:**213–233.
26. **Vargas, R., and T. Hattori.** 1990. Protozoan predation of bacterial cells in soil aggregates. FEMS Microbiol. Ecol. **38:**233–242.
27. **White, D., E. A. FitzPatrick, and K. Killham.** 1994. Use of resin impregnated soil sections to assess spatial location of bacterial inocula introduced into soil. Geoderma **63:**245–254.
28. **White, D., D. A. Wright, L. A. Glover, J. I. Prosser, D. Atkinson, and K. Killham.** 1994. A partial chloroform-fumigation technique for characterisation of spatial location of bacteria introduced into soil. Biol. Fertil. Soils **17:**191–195.
29. **Wright, D., K. Killham, L. A. Glover, and J. I. Prosser.** 1993. The effects of location on protozoan grazing of a genetically modified bacterial inoculum in soil. Geoderma **56:**633–640.