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Protozoan predation and the turnover of soil organic carbon and nitrogen in the presence of plants*

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Summary. The impact of protozoan grazing on the dynamics and mineralization of ^{14}C- and ^{15}N-labelled soil organic material was investigated in a microcosm experiment. Sterilized soil was planted with wheat and either inoculated with bacteria alone or with bacteria and protozoa or with bacteria and a 1:10 diluted protozoan inoculum. ^{14}C-CO_2 formation was continuously monitored. It served as an indicator of microbial activity and the respiration of soil organic C. The activity of protozoa increased the turnover of ^{14}C-labelled substrates compared to soil without protozoa. The accumulated ^{14}C-CO_2 evolved from the soils with protozoa was 36% and 53% higher for a 1:10 and for a 1:1 protozoan inoculum, respectively. Protozoa reduced the number of bacteria by a factor of 2. In the presence of protozoa, N uptake by plants increased by 9% and 17% for a 1:10 and a 1:1 protozoan inoculum, respectively. Both plant dry matter production and shoot: root ratios were higher in the presence of protozoa. The constant ratio of ^{15}N: $^{14+15}N$ in the plants for all treatments indicated that in the presence of protozoa more soil organic matter was mineralized. Bacteria and protozoa responded very rapidly to the addition of water to the microcosms. The rewetting response in terms of the ^{14}C-CO_2 respiration rate was significantly higher for 1 day in the absence and for 2 days in the presence of protozoa after the microcosms had been watered. It was concluded that protozoa improved the mineralization of N from soil organic matter by stimulating the turnover of bacterial biomass. Pulsed events like the addition of water seem to have a significant impact on the dynamics of food-chain reactions in soil in terms of C and N mineralization.

Key words: Protozoan grazing – Organic carbon turnover – Microbial activity – Nitrogen mineralization – Microcosm – Microbial biomass

The effects of protozoan predation on soil bacteria are significant for nutrient transformations in the soil-plant ecosystem (Elliott et al. 1984). Increased mineralization of C and N following the (pulsed) addition of glucose and ammonium-N to soil microcosms in the presence of amoebae has been demonstrated (Coleman et al. 1978; Bryant et al. 1982; Woods et al. 1982). A significant enhancement of ammonium-N uptake by plants grown in soil microcosms with bacteria and protozoa compared to those with only plants and bacteria was shown by Elliott et al. (1979) and Clarholm (1985) following the addition of fertilizer N and by Kuikman and Van Veen (1989) without the addition of N. The latter showed that grazing by protozoa led to an improved plant availability of specific bacterial N, and postulated an increased turnover of N mineralization from soil organic matter.

In general, it has been shown that the activity of amoebae reduces the number of bacteria in soil. If protozoan grazing only affected bacterial numbers negatively, microbially mediated processes such as the decomposition of soil organic matter would eventually be negatively affected as well, and protozoa would only accelerate the cycling of nutrients temporarily. Since several reports in the literature (Barsdate et al. 1974; Stout 1980) as well as our own preliminary experiments have shown the opposite, i.e. grazing of bacteria by protozoa seemed to lead to a continuously increased turnover of organic matter, two mechanisms are possible: (1) protozoa use organic material other than bacterial cells or (2) protozoan grazing stimulates the overall activity of the bacterial population.

Soil provides a very heterogeneous environment for both bacteria and protozoa. Their possibilities for movement are severely restricted by discontinuities in the waterfilm, both in time and space (Stout 1980; Vargas and Hattori 1986; Bamforth 1988). Several authors (Postma and Altemüller 1990; Foster 1988) have shown the occurrence of isolated communities of either bacteria or protozoa or both. The overall microbial activity is the sum of activity in each of these communities. Protozoa have to actively move through the soil to reach bacterial communities before they can exert an effect on bacteria

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and on bacterial metabolism. Water has been shown to be the main regulating vector (Vargas and Hattori 1986). Therefore, the frequency of bacterial-protozoan interactions is not only a function of the number and the distribution of protozoa and bacteria but also of the soil moisture content. The flow of water can thus contribute by redistributing both protozoa and bacteria through the soil.

The objectives of the present study were to: (1) determine the impact of protozoan predation of bacteria on the rate of C and N mineralization from soil organic matter in the presence of plants and a fluctuating soil moisture regime and (2) study the effect of the number and distribution of protozoa in soil. Soil labelled with ^{15}N-^{14}C-organic material was planted with wheat, which served as an N sink to accumulate information on N mineralization. Continuous monitoring of ^{14}C-CO_2 yielded information on the dynamics of microbial activity.

Materials and methods

Soils

The soil used was a loamy sand, as described by Kuikman and Van Veen (1989). It was sampled from a field plot 4 months prior to the start of this incubation and sieved (⌀ 4 mm). The soil was sterilized by exposure to a ^{60}Co-γ-radiation source (4.10^4 Gy). An incubation experiment was carried out as described elsewhere (Kuikman et al. 1990) to assess the impact of protozoan grazing on bacteria as a function of protozoan movement and the number of protozoa inoculated. Therefore, the soil was amended with bacteria which were grown on a mineral medium containing ^{14}C[U]-glucose and ^{15}N-ammonium, and part of the soil was also inoculated with protozoa. The soil that was left after taking out subsamples for several analyses was stored at 4 °C.

This ^{14}C-^{15}N-labelled soil (349 Bq×g^{-1} dry soil) was then mixed and air-dried to a moisture content of 7.7% (v:w). Nine portions of each 900 g dry soil were weighed into double plastic bags and again sterilized as described before.

The sterility of the soil was tested by preparing a dilution series of suspended soil and checking for bacterial growth on 1:2 Tryptone Soya Agar (Oxoid, U.K.) plates and for protozoa on water agar plates supplemented with a bacterial suspension; no bacteria or protozoa were found. The soil contained 45.5 µg NH_4-N and 20.2 µg NO_3-N per gram dry soil at the start.

Bacteria and protozoa

Pseudomonas fluorescens R2f was isolated from grassland soil in The Netherlands (Van Elsas et al. 1988) and *Enterobacter cloacae* was isolated from soil in South Australia. Both bacteria were grown in a mineral medium [in g l^{-1}: 4.0 glucose, 2.88 citric acid, 1.07 NH_4Cl, 0.5 K_2HPO_4, 0.5 KH_2PO_4, 0.2 $MgSO_4$, 0.01 $MnSO_4$, 0.01 NaCl, 0.01 $FeSO_4$, 0.01 $CuSO_4$, 0.00004 $ZnSO_4$, 0.00003 $CoCl_2$, 0.00002 $(NH_4)_6Mo_2O_4$] on a rotary shaker at 29 °C. Bacterial cells were harvested by centrifugation at 10000 g for 10 min at 10 °C and resuspended in sterile demineralized water. The centrifugation and resuspension was repeated once.

The protozoan population that was used consisted of one flagellated and one amoebal species. *Acanthamoeba* sp. was obtained from Dr. E.T. Elliott (NREL, CO 80523 Ft Collins, USA) and has been described by Elliott and Coleman (1977); the flagellate was identified as a *Cercomonas* sp. and was isolated from the loamy sand soil which was used in this investigation. Both protozoan species were cultured on water agar and *Pseudomonas* R2f as a food source. The protozoa were harvested by washing them from the agar plates with amoeba saline (Page 1967).

Experimental design and inoculation

The experiment was carried out in a completely randomized design with nine experimental units randomly allocated to three treatments, i.e. no protozoa, a 1:10 diluted and an undiluted protozoan inoculum. The soil portions, each 900 g (7.7% water), were inoculated with a suspension that introduced 1.7×10^7 bacteria and either 36.8×10^3 flagellates and 0.7×10^3 amoebae or a 1:10 diluted protozoan inoculum or sterile demineralized water without protozoa. After inoculation of the soil portions in plastic bags, they were thoroughly mixed by hand and aseptically transferred to the sterile microcosms. This experimental unit (polyvinyl chlorde column, 1.5 l, ⌀ 9 cm, length 24 cm) was half-filled with glass beads and half with soil. The soil core was brought to a bulk density of 1.3 g cm^{-3} (dry weight). Each soil microcosm was planted with one 10-day-old wheat (*Triticum aestivum* cv. *Ralle*) seedling. The seeds were surface-sterilized with 1.5% sodium hypochlorite and individually germinated on 1:10 Tryptone Soya Agar (Oxoid, U.K.) in 20-cm tubes. Only those seedlings were used that were not contaminated, as evidenced by bacterial or fungal growth on the medium.

The final soil moisture content at the start of the incubation was 15% (v:w), which corresponds to a water potential of approximately −10 kPa. This figure is based on moisture retention curves of the same soil treated equally as made by Postma et al. (1989). Losses of water due to evapotranspiration by plants were made up by regular additions of sterile demineralized water on top of the soil cores. Weight increase due to plant growth was taken into account. The soils were incubated in a climate chamber (day/night regimen: light 16 h at 21 °C and dark 8 h at 15 °C) for 34 days.

The soil in the column was separated from the atmosphere by a seal of plastic silicone rubber placed at the stem-base of the plants after 4 days of growth on the microcosms. Necessary openings were provided for the addition of water and for flushing the columns with sterile, CO_2-free air (inlet at the top, outlet at the base of the column).

Sampling procedure and analyses

After cutting off the shoots, the roots were separated from the soil and washed on a fine (⌀ 0.5 mm) sieve. Dry shoot and root mass was obtained by weighing the plant material after drying at 80 °C for 48 h. The plant N content was measured as ammonium-N after digestion with sulphuric acid and salicylic acid on an autoanalyser using ground (⌀0.5 mm) plant material. After separating the roots from the soil, the soil was subsampled for determination of bacterial and protozoan numbers, mineral (NH_4-N and NO_3-N) N, ^{14}C in soil organic matter, and soil moisture content.

The number of bacteria was determined by extracting 10 g fresh soil in 95 ml of 0.1% sodium pyrophosphate solution containing 10 g gravel on a rotary shaker (10 min, 200 rpm). A series of 10-fold dilutions in sterile demineralized water was prepared and appropriate dilutions were plated (in triplicate) on King's B agar [in g l^{-1}: 20 proteose peptone; 1.5 K_2HPO_4; 1.5 $MgSO_4 \cdot 7\ H_2O$; 10 glycerol; 15 agar; 1000 H_2O; pH 7.2]. After an incubation of the plates for 48 h at 29 °C, the total number of colony-forming units on each plate was determined.

The number of protozoa was determined by a modified, most-probable-number method (Darbyshire et al. 1974; Rowe et al. 1977), extracting 5 g fresh soil in 100 ml of Amoebae Saline, using fourfold dilutions and eight replicated series with *Pseudomonas fluorescens* as a food source. The microtiter plates were incubated at 12 °C in the dark and scanned for the presence of protozoa several times during a 4-week period.

After extraction of 25 g fresh soil in 50 ml 0.5 *M* K_2SO_4, mineral N, i.e., NH_4-N using Nessler's reagent (van Ginkel and Sinnaeve 1980) and NO_3-N was measured by continuous flow analysis.

The CO_2 evolved from the soil was trapped in 0.5 *M* NaOH. The soil columns were flushed with CO_2-free air (10 l h^{-1}) for 15 min four times a day. The evolution of ^{14}C in CO_2 was measured every day. The total C and ^{14}C content in dry soil and plant material were determined after combustion (Amato 1983). Total CO_2-C released by incubation or by combustion of soils was determined by titration and ^{14}C-CO_2 by liquid scintillation counting (Amato 1983) using Ultima Gold as a scintillation liquid (Packard, UK).

Statistics

For each treatment, three replicated microcosms were destructively sampled after 33 days of incubation. Analyses for determination of bacterial and protozoan numbers, soil mineral N, ^{14}C in soil organic matter and soil moisture content were made in duplicate. The results were analysed by analysis of variance over the experimental factor, i.e., the number of protozoa inoculated at the start. The accumulated ^{14}C and total CO_2 evolution was analysed by analysis of variance. These results were also analysed by fitting a response function for each microcosm. The model used was a line plus exponential curve:

$$Y = a + (b \times r^{\text{day}}) + c \times \text{day} \quad (1)$$

where $r \leqslant 1$ and the limit $(b \cdot r^{\text{days}}) = 0$. The curve passes through the origin (0.0) if $[a = -b]$. The parameter r determines when the behaviour of the curve becomes linear and the parameter c gives the slope of the linear part of the curve. The estimated parameter values were analysed by analyses of variance over the experimental factor. All differences reported are significant at the level of $P < 0.05$ at least.

Statistical analyses were performed with the statistical package GENSTAT 5, release 1.3 (Genstat 5 Committee 1987) on a VAX VMS mainframe computer.

Results

Microbial numbers

At the end of the incubation, significantly less bacteria (Table 1) were found in soils with protozoa (0.76×10^8) compared to the soils without protozoa (1.30×10^8). The number of bacteria increased approximately 5 and 10 times in soils inoculated with protozoa and in soils without protozoa, respectively. The different sizes of the protozoan inoculum did not affect the number of bacteria nor the number of protozoa that were recovered after 33 days. Approximately 3000 amoebae per g dry soil were recovered (Table 1), which is 4–40 times more amoebae than were inoculated. No flagellates were recovered. Minimal numbers of protozoa could be detected in samples from two out of there microcosms that had not been inoculated with protozoa.

Plants

The total plant dry mass production was 2.37 g dry weight per microcosm in soils with no protozoa added and significantly increased by 40% and 47% when protozoa had been added to the soils (Table 2). Shoot mass but not root mass was positively ($P < 0.01$) affected by the presence of protozoa; there was 65% more shoot mass in soils with protozoa compared to soils not inoculated with protozoa (Table 2). The consequences for the shoot : root ratio, then, are obvious: it is higher in the presence of protozoa than in the absence of protozoa (Table 2).

The final N concentration of plants, grown in soils without protozoa, was 33% (shoots) and 45% (roots) higher than in soils with protozoa (Table 2). In none of the soil microcosms could any mineral N, either ammonium or nitrate, be detected at the end of the incubation. The total plant N uptake was lowest in soils without protozoa (47.95 mg N) and significantly improved in soils inoculated with protozoa; the values were 52.24 and 55.96 mg N in soils with a diluted and a non-diluted protozoan inoculum, respectively (Table 3). The ^{15}N plant uptake followed the results for total N (Table 3). The ratio $^{15}N : {}^{14+15}N$ indicated that in soils with the undiluted protozoan inoculum, relatively less labelled N was taken up by the plants compared to the other soils (Table 3).

Soil organic C dynamics

Unfortunately, the microbial metabolization of ^{14}C, ^{14}C-CO_2 evolution and the protozoan response could not be monitored during the first 4 days of the incubation since the plants were too fragile to be sealed at the stem base.

The rate of CO_2 evolution was not significantly affected by the presence of protozoa or by the number of protozoa inoculated at the beginning of the incubation, and was highly variable during the overall incubation period (Fig. 1). The differences in accumulated CO_2 respiration varied from 450 to 480 µg C g^{-1} dry soil and were

Table 1. Numbers of bacteria and protozoa per gram dry soil (means of three replicated microcosms)

Treatment	Bacteria	Protozoa
B	1.30×10^8	–
Bp	0.76×10^8	2.77×10^3
BP	0.76×10^8	3.01×10^3
LSD ($P = 0.05$)	0.47×10^8	1.75×10^3

B, bacteria only; Bp, bacteria plus 1 : 10 diluted protozoan inoculum; BP, bacteria plus undiluted protozoan inoculum; LSD, least significant difference

Table 2. Plant dry mass (shoots, roots, total), shoot : root ratio, and plant N concentration (means of three replicated microcosms) and least significant differences ($P = 0.05$)

Treatment	Plant dry mass (g)				Plant N (%)	
	Shoot	Root	Total	Shoot : root ratio	Shoot	Root
B	1.41	0.96	2.37	1.54	2.49	1.45
Bp	2.28	1.05	3.33	2.20	1.83	1.02
BP	2.39	1.09	3.48	2.21	1.91	0.97
LSD	0.33	0.34	0.58	0.56	0.43	0.27

See footnote to Table 1

Table 3. Total plant N, plant ^{15}N uptake, ^{15}N concentration in plant N, and the ratio ^{14}C-CO_2 : ^{15}N-plant (means of three replicated microcosms)

Treatment	Plant N (mg)	Plant ^{15}N (mg)	$^{15}N : {}^{14+15}N$ (%)	$^{14}C : {}^{15}N$ (Bq mg^{-1})
B	47.95	0.557	1.161	17.8
Bp	52.24	0.613	1.173	22.0
BP	55.96	0.627	1.119	24.2
LSD ($P = 0.05$)	4.19	0.059	0.042	

See footnotes to Table 1

not significantly different at any specific time during the incubation period (Fig. 1).

The rate of ^{14}C-CO_2 evolution decreased in all treatments from day 5 until the end of the incubation (Fig. 2). The rate from soils inoculated with protozoa was consistently higher than from soil inoculated only with bacteria. However, only a few sampling days showed statistical differences in the ^{14}C-CO_2 evolution rate between soils with and soils without protozoa. Where differences occurred, the rate of ^{14}C-CO_2 evolution in soils with protozoa was significantly higher than in soils without protozoa (Fig. 2).

The accumulated ^{14}C-CO_2 that evolved from the soils without protozoa amounted to 9.9 Bq g^{-1} dry soil compared with 13.5 and 15.2 Bq g^{-1} dry soil from the soils with the 1 : 10 diluted protozoan inoculum and the undiluted protozoan inoculum, respectively (Fig. 2). Protozoa therefore significantly stimulated ^{14}C-CO_2 evolution.

The fitted response function for each microcosm accounted for at least 99% of the variance in accumulated ^{14}C-CO_2 evolution. Only the estimates for the parameter c (Eq. 1) were significantly different at 0.21 for soil without protozoa, 0.40 for soil with protozoa, and 0.38 for soil with a 1 : 10 diluted protozoan inoculum. The parameter c determines the slope of the linear part of the curve over the last 20 days of the incubation and was approximately twice as high for soils with protozoa compared with those without protzoa.

The fitted response functions for total C respiration from the individual microcosms accounted for at least 98% of the variance. Analyses of variance on the parameter estimates for the curves showed no statistical significant difference with respect to the inoculation of protozoa applied. Since the estimate for r was almost 1.0, the dynamics of CO_2 evolution could be best described by a linear relationship.

The rate of ^{14}C evolution peaked directly after the addition of water (Fig. 2) to the soil columns. The rewetting response was defined as the difference in the ^{14}C-CO_2 evolution rate (Bq day^{-1} g^{-1}) 5 days after the addition of water and the rate over the remaining 21 days of the incubation period when the level of ^{14}C-CO_2 evolution had stabilized (Fig. 3). The rewetting response was tested by analysis of variance. The 'averaged' rate of ^{14}C-CO_2 evolution was stimulated ($P<0.01$) by the addition of water. The rewetting response was significantly differ-

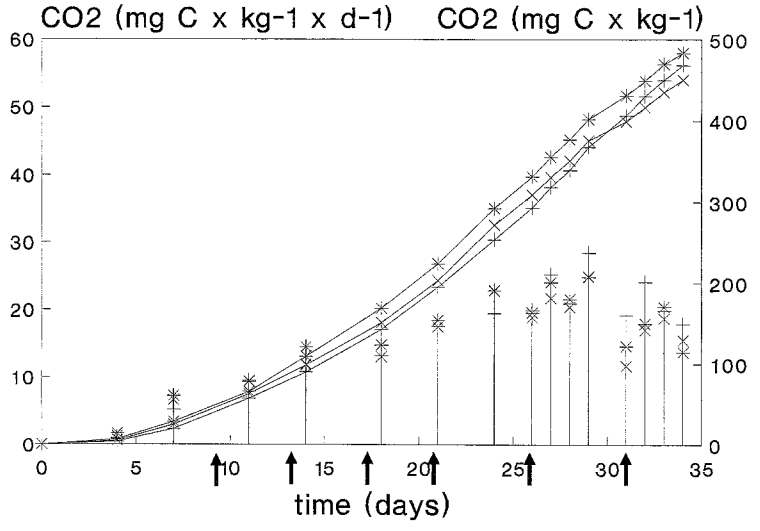

Fig. 1. Rate (*bars*) of soil respiration (mg C-CO_2 day^{-1}) and accumulated (*lines*) CO_2 evolution (mg C-CO_2 $microcosm^{-1}$) from the soil (means of three replicated soil microcosms). Water added on days 8, 12, 17, 21, 26, and 31, as indicated by the arrows: 20, 30, 40, 50, 60, and 60 ml, respectively; + no protozoa, × 1 : 10 diluted protozoan inoculum, * diluted protozoan inoculum

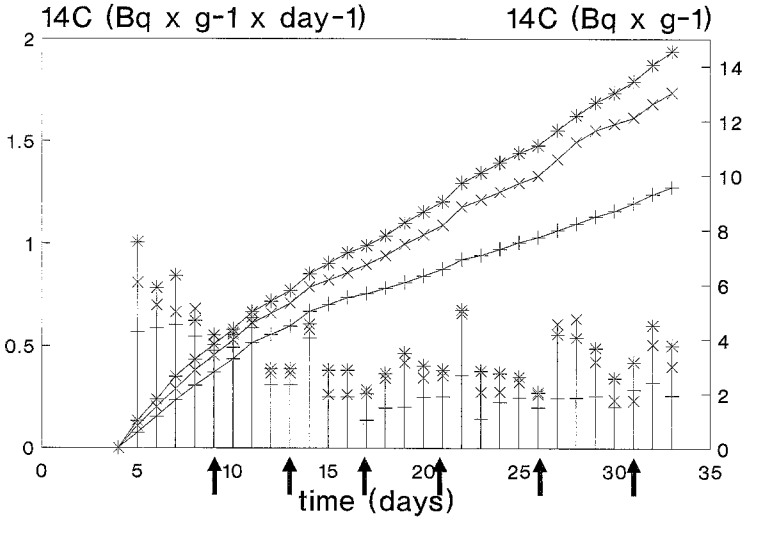

Fig. 2. Rate (*bars*) of ^{14}C-CO_2 evolution (Bq g^{-1} day^{-1}) and accumulated (*lines*) ^{14}C-CO_2 evolution (Bq g^{-1}) from the soil (means of three replicated soil microcosms). For other explanations, see Fig. 1

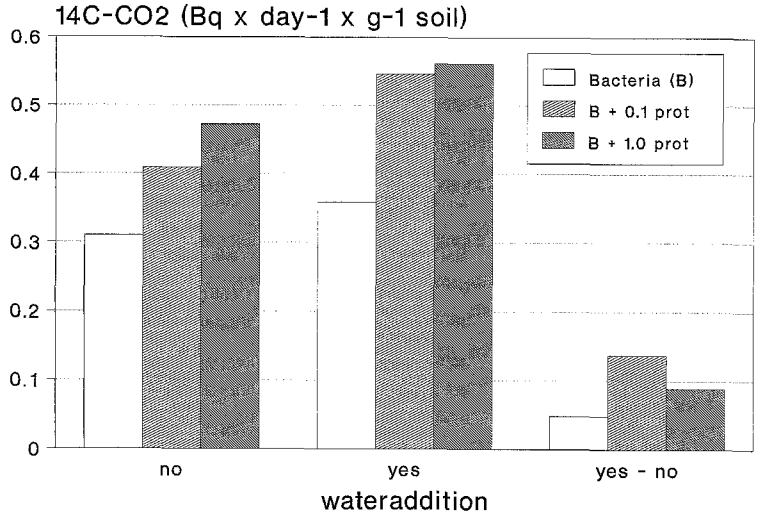

Fig. 3. Averaged rate of ^{14}C-CO_2 evolution (Bq day^{-1} g^{-1}) for 21 days not immediately following the addition of water (*no*) and for 5 days immediately following the addition of water (*yes*), and the rewetting response (difference between *yes* and *no* water addition). Means of three replicated microcosms are shown

ent among soil treatments and increased by 60%, from 0.049 to 0.078 ($P<0.01$), for the soils with the undiluted protozoan inoculum, and by 180%, to 0.137 ($P<0.01$), where only the 1 : 10 diluted protozoan inoculum was applied, compared to soils without added protozoa. The rewetting response was also investigated by comparing the ^{14}C-CO_2 evolution rate 2 days after the addition of water with the rate for the remaining days. This calculation showed that the rewetting response dropped to 0.002 in the soils without protozoa but was significantly ($P<0.05$) increased to 0.090 in the soils with the 1 : 10 diluted inoculum and to 0.055 in those with the undiluted protozoan inoculum.

Discussion

By conducting an experiment in which plants were grown on soil that contained ^{14}C- and ^{15}N-labelled organic matter, both in the absence and in the presence of protozoa, we were able to simultaneously determine the turnover of C and of N from soil organic material as affected by protozoan predation of bacteria. The activity of protozoa increased the turnover of soil organic ^{14}C as evidenced by a higher cumulative ^{14}C respiration as well as an ongoing higher ^{14}C respiration rate in the soils with protozoa. During the last 15 days of the incubation, the presence of protozoa almost doubled the microbial respiration rate. The overall respired soil organic C was 36% and 53% higher in the soils with the 1 : 10 diluted and the undiluted protozoan inoculum, respectively. These results agree with observations by Coleman et al. (1978) that soils with food chains that included bacterial grazers showed higher respiration rates compared to soils without bacterial grazers, i.e., amoebae and/or nematodes. Total soil respiration, from roots and microorganisms, was not affected by the presence or absence of protozoa and neither was the root production.

The reduction in the number of bacteria in the soils with protozoa compared to those without protozoa, even though smaller, confirmed earlier results on the predatory activity of protozoa (Clarholm 1981; Bryant et al. 1982; Kuikman and Van Veen 1989).

An increased plant mass production, and a relatively larger shoot production as indicated by an increased shoot : root ratio, in the presence of protozoa, pointed to an improved plant nutrient supply, as shown by Davidson (1969). Plant N availability was stimulated in the presence of protozoa, as shown by a higher N uptake of 9% and 17% in the soils with the 1 : 10 diluted and with the undiluted protozoan inoculum, respectively. These results confirm reports by Elliott et al. (1979), Clarholm (1985), Kuikman and Van Veen (1989) and Kuikman et al. (1989).

The plant uptake of ^{15}N corresponded to the total N uptake, as shown by the similarity of the ratio between both N sources, ^{15}N and ^{14}N, in the plants. Therefore it is concluded that the presence of protozoa affected the quantity and not the quality of the soil organic N that becomes available to plants as it is mineralized. This finding is in contrast to results by Kuikman and Van Veen (1989), who studied the turnover of ^{15}N-labelled bacterial cells. In their study, relatively more bacterial ^{15}N than ^{14}N was mineralized and recovered in plants in the presence of protozoa. The relatively low ratio of ^{15}N: $^{14+15}N$ in the soils treated with the undiluted protozoan inoculum might be explained by a relatively large contribution by non-labelled, introduced bacterial biomass N, due to immediate action by the protozoa upon their introduction. When less protozoa were introduced, with the 1 : 10 diluted protozoan inoculum, there was a less rapid turnover of unlabelled introduced bacterial N. This reasoning could also explain the relatively large contribution of labelled N from introduced labelled bacterial cells in the presence of protozoa found by Kuikman and Van Veen (1989).

The impact of protozoa on the mineralization of ^{14}C was relatively larger than the impact on mineralization of ^{15}N and plant N uptake, as indicated by the ratio ^{14}C-CO_2: ^{15}N-plants (Bq mg^{-1} ^{15}N; Table 3). As a result of the predatory activity of protozoa, nutrients are returned to the soil solution, i.e. C as a waste product of protozoan metabolism and excess NH_4^+-N (Anderson et al. 1981). Recirculation of this C and N within the bacterial-protozoan population can be expected; after being respired C

diffuses entirely from the soil whereas N can be re-used. Thus protozoa reduce bacterial numbers and thereby accelerate the turnover of bacterial C.

The rate of ^{14}C-CO_2 respiration responded markedly to the addition of water. Several authors have emphasized the potential impact of a fluctuating soil moisture content on microbial activity in soils (Stout 1973; Elliott et al. 1988). After dried soils are remoistened, an increased biological activity, as expressed by increased CO_2 production and N mineralization, has been generally accepted. The observed flushes are attributed to an increased availability of water-soluble substrates to the microorganisms, the disruption of aggregates, and the rearrangement of soil components in soils (Birch 1960; Adu and Oades 1978; Orchard and Cook 1983; Van Veen et al. 1985).

In our system, soil moisture fluctuations are created by the evapotranspiration of plants. The intensity of these fluctuations increases during the incubation period. Following the first three additions of rather modest amounts of water, no significant changes in the rate of ^{14}C respiration were detected. The final three additions of water, on days 21, 26, and 31, increased the soil volumetric water content by 6%, 7.2%, and 7.2%, respectively, which brings the water potential from approximately -250 to -10 kPa (Postma et al. 1989). These pulsed events can be considered as rainfall events. They provoked a clear and significant increase in the ^{14}C respiration rate by the microbial population which lasted 1 day in the soils without and 2 days in the soils with protozoa, as can be concluded from the calculated rewetting response. The very rapid reaction of protozoa to the addition of water, within 1 day, confirms results presented by Elliott et al. (1986) and Hunt et al. (1989), who showed that protozoa responded quickly to pulsed rainfall events, with a fivefold increase in numbers within 1 or 2 days. Kuikman et al. (1989) showed that protozoa increase the availability of bacterial N to plants, even with strong fluctuations in the soil moisture content compared with a fairly stable soil moisture regime. Thus, pulsed events do, indeed, have a significant influence on the dynamics of soil food-chain interactions in terms of C and N dynamics.

The number of protozoa inoculated did not significantly affect N availability to plants nor did it affect the final number of protozoa recovered from the inoculated soils. Previous results have shown that in unplanted soil, protozoa hardly move actively through the soil matrix. Vargas and Hattori (1986) concluded from experiments with varied numbers of inoculated protozoa that migration of protozoa among aggregates in soil was determined by the continuity of the water film connecting adjacent aggregates. However, in the present study the rewetting response (Fig. 3) was more pronounced in the soils treated with the 1:10 diluted protozoan inoculum compared to the undiluted protozoan inoculum. It appears likely that flows of water strongly affect the distribution of protozoa, so that in the 1:10 diluted protozoan inoculum treatment, ^{14}C-labelled bacteria were available to the protozoa for a relatively longer time compared to the undiluted protozoan inoculum treatment in which the high initial number of protozoa reduced the number of bacteria and their activity to a greater extent initially. In the undiluted protozoan treatment, regrowth of bacteria occurred in places already inhabited by protozoa, whereas in the 1:10 diluted treatment, after the induced waterflow, the protozoa were able to inhabit new places not reached before.

Kuikman and Van Veen (1989) have postulated that the mechanism by which the protozoa stimulate N mineralization from soil organic matter is an increased turnover of bacterial biomass. The present data confirm this hypothesis by showing that protozoa do simultaneously stimulate the turnover of soil organic C and improve N mineralization and N availability to plants.

There has been discussion on whether stimulation by protozoa should be attributed to direct consumption of bacteria and the subsequent release of excess nutrients or to the indirect mechanism of increased bacterial activity, or both (Barsdate et al. 1974; Anderson et al. 1981). Our results have shown that in the presence of protozoa, up to 50% more ^{14}C and 20% more ^{15}N was mineralized than in soils with twice as many bacteria but without protozoa. Therefore bacterial cells would have to respire twice as much ^{14}C-CO_2 in the presence of protozoa to be comparable with the results from soils without protozoa. However, if the elevated ^{14}C-CO_2 respiration is due to direct consumption of 50% of the bacterial cells by protozoa, theoretically the protozoa would have to respire all of the ^{14}C ingested to increase the ^{14}C-CO_2 respiration by 50%, which is unlikely (Anderson et al. 1981). Thus, it appears that a bacterial population, even though reduced in numbers, is more active in terms of the amount of C metabolized per unit biomass when being preyed upon by protozoa. In conclusion, protozoa not only accelerate the turnover of C and N by reducing the size of the bacterial population but also seem to increase the activity of the bacterial population in soil.

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