# INTERACTIONS OF BACTERIA, PROTOZOA AND PLANTS LEADING TO MINERALIZATION OF SOIL NITROGEN

## MARIANNE CLARHOLM

Department of Microbiology, Swedish University of Agricultural Sciences, S-750 07 Uppsala, Sweden

**Summary**—The capacity of bacteria and protozoa to mineralize soil nitrogen was studied in microcosms with sterilized soil with or without wheat plants. The effect of small additions of glucose or ammonium nitrate or both, twice a week was also tested. Plant dry weight and N-content, number of microorganisms and biomass plus inorganic N were determined after 6 weeks.

The introduction of plants profoundly influenced the N transformations. In the presence of root-derived carbon, much more N was mineralized from the organic matter and immobilized mainly in plant biomass. "Total observable change in biomass N plus inorganic N" was negative in the unvegetated soils without additions, while a mineralization of 1.7 mg N microcosm$^{-1}$ was observed in microcosms with wheat plants grown with bacteria only. When protozoa were included, the N taken up by plants increased by 75%. Sugar additions resulted in an 18% increase of total N in the shoots when protozoa were present, but had no significant effect in the absence of grazers. Plants with the same root weight were more efficient in their uptake of inorganic N when protozoa were present. Plants grown with protozoa also had a lower R/S ratio, indicating a less stressed N availability situation. The lowest ratio was found with N additions in the presence of protozoa.

The results indicate that, with energy supplied by plant roots or with external glucose additions, soil bacteria can mineralize N from the soil organic matter to support their own growth. Grazing of the bacteria is necessary to make bacterial biomass N available for plant uptake.

## INTRODUCTION

Most nitrogen mineralization studies published so far have been performed by incubating fallow soil in the field or in the laboratory under standardized temperature and moisture conditions (Stanford, 1982), or with different moisture and temperature regimes (Clarholm *et al.*, 1981). In these experiments, the amount of inorganic N present at the end of the incubation has been used to estimate the size of the mineralization. For two reasons, it is difficult to relate the results from such studies to the vegetated field situation. Firstly, the bacterial activities leading to N mineralization would have been depressed because of the lack of a suitable energy source, which is supplied by plant roots in the vegetated soil. Secondly, without root uptake, unnaturally high amounts of inorganic N will build up in the soil, possibly retarding further mineralization. In field studies, Bartholomew and Clark (1950) found, using $^{15}$N, that the total mineralization of N in cropped soils was much higher than in fallow soil. They also estimated that the total microbial activity was roughly four times greater in the vegetated soil.

To be active, bacteria are usually dependent on an easily-available energy source. Lynch (1976) examined the evidence in several investigations estimating bacterial growth rates and concluded "that there is little or no growth of the populations in the bulk soil, and that the rhizosphere and the area around crop residues are the only sites of active growth". By means of a selective inhibition technique, Vančura and Kunc (1977) showed that bacteria contributed more than fungi to the respiration in the rhizosphere,

while the reverse was true in the bulk soil. It therefore seems likely that the bacterial mineralization of N in the vegetated field soil will be underestimated in investigations without plant roots or an added suitable energy source.

In a number of experiments, Coleman and co-workers (Coleman *et al.*, 1977; Woods *et al.*, 1982) followed the development of bacteria, bacterial grazers and inorganic N in microcosms with sterilized soil after an initial addition of glucose. Adding sugar caused an immobilization of added inorganic N in bacterial biomass. This N was later released through grazing by protozoa and nematodes. The added sugar was sufficient to make the bacteria immobilize all the N present at the start, but not enough to sustain prolonged bacterial growth, which would require a supply of additional C. Even in a later experiment where C was added more than once (Bryant *et al.*, 1982), the N demand of the bacteria could be satisfied by the N released by grazing, since no roots were present to withdraw inorganic N from the soil solution.

The above mentioned microcosms may be viewed as models of the events occurring in a specific volume of soil when it is under the temporary influence of a root (Coleman *et al.*, 1978). The C addition, which may be considered equivalent to the input of C from the root, results in bacterial growth followed by growth of bacterial grazers, leading to the release of inorganic N. Here the resemblance to natural conditions ends, since roots for the N-uptake were lacking.

Elliott (as cited by Anderson *et al.*, 1981) has suggested a series of events on the root surface, starting with an output of root-derived carbon just

behind the apex followed by bacterial growth resulting in N immobilization and subsequent mineralization through protozoan grazing. The inorganic N was assumed to enter the rhizosphere through diffusion from an unidentified source. Another possibility, which I have explored, is that bacteria can release N from the organic matter, when supplied with a suitable source of energy. The experiment was set up to investigate the role of root-derived carbon for the bacterial-mediated N mineralization, and if protozoan grazing is necessary to make the bacterial N available for root uptake.

## MATERIALS AND METHODS

*The experimental design* contained in all 16 alternatives, each with 4 replicates. Sterilized soil with reintroduced bacteria was incubated with or without protozoa, with or without plants. These 4 treatments were in turn exposed to 4 sets of repeated additions: water only, water with a C source, water with an N source, or water with both C and N.

*The microcosms* consisted of large test-tubes (200 × 25 mm) each filled with 35 g air-dried soil, which had been stored at field moisture at 2°C for 6 months. The soil was sieved (<4 mm), dried at room temperature and then wetted to field capacity. The test-tubes were plugged with cotton wool and autoclaved twice, with an interval of 2 days between autoclavings.

*The soil* used was a top soil from the experimental field of the "Ecology of Arable Land" project. The top soil is a loam, 27 cm thick; 6.6% loss on ignition; 0.25% N; pH 6.3 (Steen et al., 1983).

*Bacteria* for inoculation were prepared by mixing soil with water in a kitchen mixer (Braun) and then filtering the water phase twice through a 3 μm Nucleopore filter. The filtrate was allowed to stand for 24 h so that any small contaminating flagellates would multiply and be detectable by microscopic examination. Bacteria were counted by epifluorescence microscopy at 1000 times magnification after staining with acridine orange (Clarholm and Rosswall, 1980). Biomass was calculated from size-class estimations (Clarholm and Rosswall, 1980) assuming a dry weight of 0.2 of the wet weight and a density of 1.0 of the dry weight.

*Protozoa* for inoculation were obtained by collecting protozoan cysts from several wells of microtitration plates previously used for enumerations of protozoa in the same soil (Clarholm, 1981). The sizes of the inocula added to each microcosm in 1 ml water were $4 \times 10^5$ bacteria, $3 \times 10^3$ amoebae and $3 \times 10^3$ flagellates $g^{-1}$ dry wt soil; ciliate numbers were below the detection limit ($\simeq 50 \ g^{-1}$). Protozoa were counted by a most probable number method (Darbyshire et al., 1974) using microtitration plates. Amoebae saline (Page, 1967), mixed with washed growing bacteria originating from the same soil, served as food source for the protozoa.

*The dry weights* used for biomass calculations were: 1 ng for amoebae (Band, 1959; Coleman et al., 1980), 260 pg for flagellates (Fenchel, 1982) and 1.4 ng for ciliates (Curds and Cockburn, 1968). An N content of 5% of the dry weight was assumed for bacteria and protozoa.

*Wheat seed* were surface-sterilized with ethanol and, after germination on nutrient agar plates, three seedlings were planted in each microcosm. The microcosms were inoculated and planted on the same day (day 1). The experiment was run in a growth chamber at 15°C, and the daily dark–light cycle was 8–16 h.

*The level of C additions* was determined in a separate experiment where glucose to obtain concentrations between 0 and 26.4 mg ml$^{-1}$ soil water (0–8000 μg g$^{-1}$ dry soil basis) was added to pots with 4.7 kg unsterilized soil and 52 wheat seedlings. The experiment was run at 60% of water-holding capacity for 5 weeks and glucose was added twice a week. Dry weight and N content of the shoots and dry weight of the roots were determined at the end of the experiment.

The 250 μg g$^{-1}$ additions resulted in 17% higher plant dry weights and 4% higher N contents than the plants without sugar additions, and also in 15% higher dry weights and 26% higher N contents than the plants with the 500 μg g$^{-1}$ additions. A level of 175 μg g$^{-1}$ was chosen since it would certainly be beneficial, and possibly more so than the 250 μg g$^{-1}$, taking the large decrease in N contents between 250 and 500 μg g$^{-1}$ into consideration.

*N additions* were then made as 0.25 of the C concentrations. Additions of 1.2 mg C (175 μg g$^{-1}$ dry soil basis) as glucose and 0.3 mg N as NH$_4$NO$_3$ were made to the respective microcosms of the main experiment twice a week for 6 weeks. In all, 14.4 mg C and 3.6 mg N were added. All additions were sterile.

*At the sampling* at the end of the experiment, the test-tubes were wrapped in a towel and gently broken with a hammer. The plant shoots were cut off, the soil with roots was thoroughly mixed, and subsamples for organisms and inorganic N determinations were taken. The roots were then collected on a sieve by washing. Dry weight of the plant and water content of the soil were determined after drying at 85°C.

*Inorganic N* was extracted by shaking the soil with 2 M KCl for 2 h. NO$_3^-$ and NH$_4^+$ were determined by flow injection analysis (Gine et al., 1980) using an FIA 06 Flow Photometer (Bifok, Sollentuna, Sweden). Total N in seeds, plant shoots and roots were determined with an automatic elemental nitrogen analyzer (Carlo Erba, Milan).

*The statistical significance* of differences was tested by analysis of variance or "Student's" t-test. Statistically significant differences reported were significant at least at the 99.9% level.

## RESULTS

### Effects on plant biomass

In all treatments, plants grown with protozoa had a larger biomass (Table 1) and contained larger absolute amounts of N in the shoots but not in the roots (Table 3). The R/S ratio did differ between treatments, with the root biomass weighing between 32 and 62% of the shoot biomass (Table 4).

### Effects on bacteria

Without protozoa, bacterial numbers did not differ significantly between treatments. With protozoa

Table 1. Weight and N-concentration of wheat plants grown in microcosms for 6 weeks with different microorganism and N or C additions. Mean and coefficient of variation (CV = standard deviation as % of the mean) $n = 4$

| Treatments | Additions | Shoots (mg dry wt microcosm$^{-1}$) | Shoots N (%) | Roots (mg dry wt microcosm$^{-1}$) | Roots N (%) | Inorganic N NH$_4^+$ ($\mu$g g$^{-1}$) | Inorganic N NO$_3^-$ ($\mu$g g$^{-1}$) |
|---|---|---|---|---|---|---|---|
| — | None | | | | | 4.9 (27) | 0.1 (0) |
| — | 14 4 mg C | | | | | 5.1 (14) | 2.9 (65) |
| Bacteria | 3.6 mg N | | | | | 3.8 (34) | 0.5 (44) |
| | C + N | | | | | 7.2 (7) | 0.1 (0) |
| | Mean of treatment ($n = 16$) | | | | | 5.2 (30) | 0.5 (226) |
| — | None | | | | | 5.4 (31) | 1 4 (11) |
| Protozoa | 14.4 mg C | | | | | 4.2 (31) | 2.7 (3) |
| Bacteria | 3.6 mg N | | | | | 4.3 (11) | 3 1 (5) |
| | C + N | | | | | 6.9 (16) | 3.5 (9) |
| | Mean of treatment ($n = 16$) | | | | | 5.2 (30) | 2.3 (38) |
| Wheat | None | 115 (17) | 3.4 (11) | 68 (25) | 1.4 (10) | 1.8 (42) | 2.4 (5) |
| — | 14.4 mg C | 118 (18) | 3.6 (4) | 69 (26) | 1.2 (14) | 0.4 (174) | 0 0 (0) |
| Bacteria | 3.6 mg N | 120 (6) | 4.2 (5) | 65 (46) | 1.5 (17) | 5.5 (26) | 1.2 (20) |
| | C + N | 113 (16) | 3.6 (4) | 56 (25) | 1.4 (12) | 6.0 (15) | 1.9 (60) |
| | Mean of treatment ($n = 16$) | 116 (16) | 3.7 (9) | 65 (29) | 1.4 (7) | 3.4 (77) | 1.1 (79) |
| Wheat | None | 213 (5) | 2.1 (5) | 113 (17) | 1.2 (7) | 2.9 (47) | 3.5 (4) |
| Protozoa | 14.4 mg C | 170 (21) | 3.2 (23) | 73 (60) | 1.5 (10) | 0.1 (0) | 0 0 (0) |
| Bacteria | 3.6 mg N | 193 (15) | 3.4 (15) | 63 (38) | 1 5 (16) | 0 1 (78) | 0.3 (68) |
| | C + N | 183 (7) | 3.3 (11) | 59 (46) | 1.7 (8) | 1.0 (33) | 0 1 (0) |
| | Mean of treatment ($n = 16$) | 189 (14) | 3.0 (24) | 77 (45) | 1 5 (169) | 1.0 (135) | 0 8 (178) |

present, the bacterial numbers were significantly higher with plants (Table 2). Bacterial biomass was always significantly higher in the presence of plants while protozoa did not have any effect.

The direct microscopical observations of the soil revealed that the microaggregate structure, seen in natural unsterilized soil, was not restored after autoclaving. The bacteria seemed to adhere less strongly to the particle surfaces, and fewer aggregated bacteria were also observed. The individual bacterial cells were larger, but their numbers were only half to one-third the level obtained in field estimations (Schnürer et al., 1982).

### Effects on protozoa

Flagellates and ciliates were significantly more numerous with plants than without (Table 2), while extremely high populations of amoebae were observed in the unplanted soils with additions, especially of C.

### Soil N and plant N

Microcosms with plants contained less NH$_4^+$ and less NO$_3^-$ (Table 1) as compared with the equivalent unplanted treatments. The highest level of total inorganic N, 10 $\mu$g g$^{-1}$, was recorded in the soil without plants with C + N additions and protozoa.

With protozoa present, the mineralized N taken up by plants increased by 75%, on average, for all four additions (Table 3). With plants and bacteria only, around 1.7 mg N was mineralized from the soil, of which most was immobilized by the plant. Without plants or C additions, no mineralization (observable change in biomass N plus inorganic N) could be detected (Table 3).

### Soil water content and plant dry weight

The soil water contents of the series with plants, protozoa and bacteria were only half the level of those in the three other treatments (Table 2) at the end of the experiment. The high evapotranspiration in this treatment caused an accidental drying out 1 week before the experiment was ended. The plants in the C, N and C + N additions dried out so severely that most of the shoots died and growth was minimal for the last week. Therefore, the total N contents of the plants, where those in series without additions had the lowest levels, probably provide better indications of the effect of additions than the dry weights do.

### Responses to C and N additions

Additions of C to the plants grown in soil with protozoa, resulted in a significant 18% increase of N in the shoots (calculated by multiplication of dry weights and %N contents in Table 1), while the N contents did not vary significantly without grazers. An addition of N alone resulted in significantly higher N contents of the shoots both with and without protozoa. If the N additions were made together with C, the plants were only able to utilize the N when grazers were present.

When the "total observable change in biomass plus inorganic N" (Table 3) was calculated, large losses were found in five of the eight cases where inorganic N had been added. The losses were reduced in the presence of protozoa in treatments with plants or when C had been added to the unvegetated soil. In the first case the N was immobilized in plant biomass, and in the second, in an extremely large amoebal biomass.

Table 2. Bacterial numbers and biomass, protozoan numbers and soil water contents in microcosms run for 6 weeks. Means and coefficient of variation (CV = standard deviation as % of the mean) $n = 4$

| Treatments | Additions | Bacteria | | Protozoa | | | Final $H_2O$ content (% of dry soil) |
|---|---|---|---|---|---|---|---|
| | | Numbers ($10^9$ g$^{-1}$ dry wt) | Biomass (mg g$^{-1}$ dry wt) | Amoebae ($10^4$ g$^{-1}$ dry wt) | Flagellates ($10^5$ g$^{-1}$ dry wt) | Ciliates ($10^3$ g$^{-1}$ dry wt) | |
| — | None | 1.7 (39) | 0.21 (48) | | | | 30 (2) |
| — | 14.4 mg C | 1.6 (23) | 0.30 (40) | | | | 33 (2) |
| — | 3.6 mg N | 1.4 (7) | 0.22 (23) | | | | 33 (1) |
| Bacteria | C + N | 1.3 (21) | 0.22 (23) | | | | 32 (1) |
| | Mean of treatment ($n = 16$) | 1.5 (26) | 0.23 (39) | | | | 31 (4) |
| — | None | 1.0 (25) | 0.16 (25) | 11 (73) | 5 (10) | 9 (40) | 30 (2) |
| Protozoa | 14.4 mg C | 1.3 (23) | 0.21 (38) | 260 (38) | 7 (81) | 5 (200) | 32 (1) |
| Bacteria | 3.6 mg N | 1.2 (28) | 0.21 (43) | 53 (519) | 7 (93) | 3 (41) | 33 (2) |
| | C + N | 1.4 (6) | 0.18 (67) | 270 (32) | 4 (50) | 0 (0) | 32 (1) |
| | Mean of treatment ($n = 16$) | 1.2 (21) | 0.19 (42) | 150 (91) | 5 (98) | 4 (130) | 31 (4) |
| Wheat | None | 1.3 (11) | 0.26 (12) | | | | 30 (18) |
| — | 14.4 mg C | 1.6 (29) | 0.40 (23) | | | | 25 (16) |
| Bacteria | 3.6 mg N | 1.4 (16) | 0.36 (11) | | | | 26 (9) |
| | C + N | 1.5 (11) | 0.33 (15) | | | | 33 (12) |
| | Mean of treatment ($n = 16$) | 1.5 (19) | 0.34 (24) | | | | 28 (17) |
| Wheat | None | 2.4 (13) | 0.25 (28) | 4 (126) | 10 (54) | 21 (65) | 13 (25) |
| Protozoa | 14.4 mg C | 2.2 (19) | 0.32 (9) | 5 (50) | 15 (53) | 12 (46) | 11 (30) |
| Bacteria | 3.6 mg N | 1.2 (41) | 0.23 (26) | 9 (99) | 12 (93) | 1 (518) | 19 (17) |
| | C + N | 2.0 (25) | 0.30 (13) | 8 (92) | 11 (34) | 7 (33) | 12 (48) |
| | Mean of treatment ($n = 16$) | 1.9 (35) | 0.28 (21) | 6 (94) | 12 (60) | 12 (77) | 13 (42) |

Table 3. Inorganic N and nitrogen content in organisms after 6 weeks. The microcosms held 35 g dry soil and contained 0.68 mg inorganic N after autoclaving. The N contents of the three seeds (3.24 mg) have been subtracted from the figures given for plant N. Total observable change was calculated by addition of all biomass values and subtraction of the 0.68 mg present at start and of 3 6 mg where N had been added All values are given in mg microcosm$^{-1}$. Values are derived from figures in Tables 1 and 2

| Treatment | Additions | Inorganic N | Bacterial N | Protozoan N | Plant N-seed N | Total observable change in biomass N plus inorganic N |
|---|---|---|---|---|---|---|
| — | None | 0.17 | 0 36 | | | −0.15 |
| — | 14.4 mg C | 0.26 | 0.53 | | | 0.11 |
| Bacteria | 3.6 mg N | 0.15 | 0.34 | | | −3 79* |
| | C + N | 0 26 | 0.39 | | | −3 63* |
| — | None | 0.24 | 0.28 | 0.23 | | −0.07 |
| Protozoa | 14 4 mg C | 0.24 | 0.36 | 4.66 | | 4 58 |
| Bacteria | 3.6 mg N | 0.26 | 0.36 | 0.96 | | −2.70* |
| | C + N | 0.36 | 0.31 | 4.79 | | 1.18* |
| Wheat | None | 0.15 | 0.45 | | 1.61 | 1 53 |
| — | 14.4 mg C | 0.01 | 0.70 | | 1.78 | 1 81 |
| Bacteria | 3.6 mg N | 0.23 | 0.63 | | 2.65 | −0.77* |
| | C + N | 0.28 | 0.58 | | 1.67 | −1 75* |
| Wheat | None | 0.22 | 0.44 | 0.16 | 2.55 | 2 69 |
| Protozoa | 14 4 mg C | 0.00 | 0.56 | 0.18 | 3.04 | 3.10 |
| Bacteria | 3.6 mg N | 0.41 | 0.41 | 0.22 | 4.13 | 0.89* |
| | C + N | 0.04 | 0.52 | 0.20 | 3.74 | 0.22* |

*3.6 mg N added during the experiment.

## DISCUSSION

The bacteria in microcosms without plants or C additions were severely energy-limited throughout the experiment, and little N mineralization was observed in treatments (Table 3) where the only C source was the killed microorganisms. The minimum amounts of C and N available to the bacteria through autoclaving can be calculated. The biomass of bacteria per g dry wt of the unsterilized soil was 0.20 mg dry wt. Assuming a 1-to-4 relation between the biomass of bacteria and fungi (Schnürer et al., 1982) and a fumigation factor of 0.4 (Jenkinson, 1976), the autoclaving would lead to an addition of approximately 7 mg C and 0.9 mg N per microcosm, if the often quoted C/N ratio of 8 is used.

In the microcosms with plants, the largest C addition came from the roots. The input would have been around 130 mg for 3 plants during the 42 days, if calculated on the basis of Fig. 2 in Sauerbeck and Johnen (1976). An addition of 14.4 mg C led to a mean increase of 0.44 mg N in "total observable change" (Table 3). This indicates that 130 mg root C would lead to a mineralization of about 4 mg N of which 2.55 mg was immobilized in plant biomass (Table 3, no addition, with protozoa).

Regardless of treatments, plants grown with protozoa present always contained larger amounts of N as compared with plants grown with bacteria only (Table 3). Annual plants take up most of the N during their early growth phase (Knowles and Watkin, 1931), resulting in high % N contents initially, which decrease as the plant grows. For a correct evaluation of the effect of a treatment or an addition on the N uptake, it is therefore necessary to compare total amounts of N in the plants. In the treatments where N had been added, the amounts of root biomass were the same with or without grazers (Table 1). The roots had thus been more effective in their N uptake with grazers present, which is also indicated by the lower inorganic N values (Table 1). Increased uptake of inorganic N by plants in the

presence of protozoa was also reported by Elliott et al. (1979).

In the present experiment, all or part (in the treatments with N additions) of the N incorporated into the plant, in excess of seed N, originated from the soil organic matter. The mechanism of the release may be as follows: the carbon released from the roots serves as an energy source for the normally energy-limited bacteria (Stotzky and Norman, 1963); temporarily released from their substrate shortage, the bacteria are able to mineralize N from the organic matter for their own growth; bacterial grazers, of which protozoa seem to be the most important, will consume the bacterial production; by excretion of surplus N as ammonium (Hardin, 1944; Fenchel and Harrison, 1976), the bacterial grazers make the N available for plant uptake.

Without grazers, the plants in all treatments took up about 1.7 mg N (Table 3) except when N was added alone. This N probably originated mainly from the microorganisms killed by autoclaving and was released by ammonifying bacteria. The most often used C/N ratio of 8 would give 0.9 mg N from the microbial biomass as previously calculated. Other experiments (J. Schnürer, personal communication) indicate, however, that a ratio of 4 is more appropriate for the soil used. This would lead to a release of about 1.8 mg N by autoclaving. The input from the necromass is a one-time event and the differences in N available for plant uptake are likely to have become larger over a longer experimental period.

The C or C + N additions had no effect of the plant N content without grazers. The increase in plant N content observed after C additions in the presence of protozoa could be explained by the creation of an enlarged rhizosphere effect by the "rain" of carbon. The addition caused bacterial growth everywhere in the soil followed by protozoan growth and N release. Some of this N was captured by the roots, leading to the observed increase, but it is likely that much of it was also denitrified.

Without plants, the C additions created amoebal

Table 4 Root dry weight as percentage of shoot dry weight for 3 wheat plants grown with C, N or C + N additions twice a week for 6 weeks in soil microcosms in the absence and presence of protozoa, respectively. Mean ± standard deviation, $n = 4$

| Additions | Bacteria | Bacteria + Protozoa |
|-----------|----------|---------------------|
| None      | 62 ± 23  | 53 ± 7              |
| C         | 60 ± 21  | 42 ± 19             |
| N         | 54 ± 25  | 32 ± 9              |
| C + N     | 53 ± 10  | 32 ± 13             |

populations 20 times larger than those normally found in vegetated soil (Elliott and Coleman, 1977). They can be viewed as an abnormal reaction to an abnormal situation. Without roots there is normally little suitable energy source for bacteria and thus little food production for protozoa. With roots present, the bacterial growth is enhanced but there is also competition from the roots for the released N, and N availablility will control bacterial growth. The comparatively low and normal (Clarholm, 1981) numbers of amoebae observed in the presence of roots, plus the fact that more N was immobilized by the plants (Table 3), indicate that the plant was a successful competitor.

There was a clear response to the N additions in the way the plants proportioned their growth into shoots and roots (Table 4). A lower proportion of the biomass in roots indicates higher N availability (Davidson, 1969). Accordingly, the most limited N situation for the plant must have been in the soil without protozoa and with no additions. With grazers, both C and N additions improved the plant's access to N as compared with no additions, with the smallest proportion in roots in the N additions.

The major loss of N from the microcosms (Table 3) is likely to have been through denitrification. Theoretically an N-to-C ratio of 1:1.25 is needed for denitrification (Delwiche, 1981). Total denitrification of the 3.6 mg N added would thus only need 4.0 mg C, still leaving most of the added C available for bacterial growth on other N sources. The large N loss observed in the bacteria + N treatment was probably caused by denitrifying bacteria, using the dead microbial cells from the sterilization as an energy source. By comparing plant N with and without N additions, it is obvious that most of the added N must have been lost also in microcosms with plants. Denitrifiers are more than 1000 times more common in the rhizosphere (Rouatt et al., 1960), where they are less energy-limited.

The observations of fewer but larger bacterial cells and the partial loss of the microaggregate structure of the soil indicated a different situation for the bacteria in the sterilized microcosms than for those in natural soil. This may be explained by the fact that fungi as well as the whole predator chain above protozoa were lacking, but it is also possible that the soil structure normally observed needs a longer time to develop.

The close relationships demonstrated between plants, bacteria, protozoa and soil make it questionable whether the components could be relevantly studied in isolation. Barber and Lynch (1977) found that the average bacterial generation time on sterilized roots grown in nutrient solution increased after 7 days. Their result might have been different if

protozoa, always present in soil, had been included in the experiment. The grazers would have reduced the bacterial populations on the older parts of the roots. Instead, an increasingly larger bacterial biomass would have had to share the C coming mainly from a small region behind the root tip (Schippers and van Vuurde, 1978) resulting in overall lower growth rates. In soil, physical barriers would also have restricted the diffusion of C to all parts of the bacterial population resulting in a differentiation of growth rates at different parts of the root.

Microcosms are useful tools for increasing our understanding of complicated interactions, especially if they contain all the biotic components pertinent to the questions asked. The present results show that bacteria are able to mineralize N from the soil organic matter, but only when supplied with energy from the roots or with external additions. The results also show that grazing, in this case by protozoa, is necessary for the release of N from the bacterial biomass for plant uptake. More added inorganic N can likewise be taken up by plant roots, in an otherwise identical situation, with protozoa present.

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