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Effects of Grazing by Flagellates on Competition for Ammonium between Nitrifying and Heterotrophic Bacteria in Soil Columns

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The enhanced mineralization of immobilized nitrogen by bacteriophagous protozoa has been thought to favor the nitrification process in soils in which nitrifying bacteria must compete with heterotrophic bacteria for the available ammonium. To obtain more insight into this process, the influence of grazing by the flagellate *Adriamonas peritocrescens* on the competition for ammonium between the chemolithotrophic species *Nitrosomonas europaea* and the heterotrophic species *Arthrobacter globiformis* in the presence of *Nitrobacter winogradskyi* was studied in soil columns, which were continuously percolated with media containing 5 mM ammonium and different amounts of glucose at a dilution rate of 0.007 h^{-1} (liquid volumes). *A. globiformis* won the competition for ammonium. The grazing activities of the flagellates had two prominent effects on the competition between *N. europaea* and *A. globiformis*. First, the distribution of ammonium over the profile of the soil columns was more uniform in the presence of flagellates than in their absence. In the absence of flagellates, relatively high amounts of ammonium accumulated in the upper layer (0 to 3 cm), whereas in the underlying layers the ammonium concentrations were low. In the presence of flagellates, however, considerable amounts of ammonium were found in the lower layers, whereas less ammonium accumulated in the upper layer. Second, the potential ammonium-oxidizing activity of *N. europaea* was stimulated in the presence of flagellates. The numbers of *N. europaea* at different glucose concentrations in the presence of flagellates were comparable to those in the absence of protozoa. However, in the presence of flagellates, the potential ammonium-oxidizing activities were four to five times greater than those in the absence of protozoa.

Soil N processes may be affected by the presence of bacteriophagous protozoa (3, 8). Grazing of protozoa on bacteria has often been reported to enhance the mineralization of bacterial N (6, 11, 13, 27, 32), although in some other studies no significant effects on ammonification rates by the grazing activities of soil microfauna were found (1, 2). Enhanced N mineralization may either stimulate the growth of N-limited heterotrophic bacteria and plants (7, 12, 16, 18) or increase the rate of nitrification in soils with low C/N ratios (14).

In competition experiments, which were conducted with mixed cultures of *Nitrosomonas europaea* and *Arthrobacter globiformis* in the presence of *Nitrobacter winogradskyi* but in the absence of flagellates in chemostats and continuously percolated soil columns, the nitrification process was shown to be inhibited by NH_4^+ assimilation by the more competitive heterotrophic bacteria (28, 29). Should protozoa be introduced in similar experiments, the grazing activities of the flagellates would be expected to result in the mineralization of part of the organic N immobilized in the mixed bacterial community. At low C/N ratios in the medium, the ammonium excreted by the protozoa will probably be converted into nitrate by the nitrifying community, because the heterotrophic bacteria are limited by carbon at low C/N ratios. As a result, the nitrate production at low C/N ratios is expected to be greater in the presence of flagellates than in their absence. At high C/N ratios in the medium, however, the mineralized ammonium excreted by the flagellates is expected to be used by the heterotrophic bacteria, which are nitrogen limited at high C/N ratios. Except for the increased mineralization of immobilized nitrogen, the stimulating effect of protozoa on the nitrification process may be due to an unknown growth factor, which is excreted by the protozoa in liquid cultures (14). In this report, transportation of ammonium toward the nitrifying bacteria, which are possibly growing in immobile microcolonies, or transportation of nitrifying bacteria toward their substrate, ammonium, is also suggested as a possible mechanism.

The aim of the present study was to investigate the influence of grazing by the bacteriophagous flagellate *Adriamonas peritocrescens* on the nitrate production in carbon- or nitrogen-limited mixed cultures of the chemolithotrophic species *N. europaea* and the heterotrophic species *A. globiformis* in the presence of *N. winogradskyi* in nonsaturated soil columns, which were continuously percolated with media containing 5 mM ammonium and different concentrations of glucose at a dilution rate of 0.007 h^{-1} (liquid volumes).

MATERIALS AND METHODS

Soil sampling. Soil was collected from the top layer (0 to 30 cm) of a grassland near Brummen (52°05'N, 06°09'E) in The Netherlands. The soil was sieved (2 mm) to remove roots and stones and was well mixed. It was a sandy soil containing 5.3% clay, 4.1% silt, 69.3% fine sand, and 21.3% coarse sand. The pH-H_2O of the sieved mixed soil was 7.8, the pH-KCl was 7.4, the content of calcium carbonate was 1.6%, and the organic matter content was 4.2%. Before use, the soil samples were stored at 4°C for 2 weeks.

Organisms and culture conditions. *N. europaea* ATCC 19718 and *N. winogradskyi* ATCC 25391 were used as the nitrifying bacteria in the present study. They probably are the dominant nitrifying bacteria in the grassland soil described above, as indicated by a positive reaction to specific

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antibodies against these bacteria in the most diluted, positive tubes of a most-probable-number (MPN) enumeration. The culturing of a mixed nitrifying population before inoculation into the soil columns was identical to the one described previously (28).

A. globiformis was used as the heterotrophic bacterium. It was isolated from the rhizosphere of Ribwort plantain (*Plantago lanceolata*), which was growing in a pot containing the soil described above. The isolation procedure and culturing of the heterotrophic bacterium have been described previously (29).

The flagellate species *A. peritocrescens*, which was isolated from the grassland soil described above, was used as the bacterial grazer in the present study. It was enriched on heterotrophic bacteria growing on 0.02% (wt/vol) proteose peptone, isolated by a dilution method, and grown monoxenically on *A. globiformis*.

Competition experiments. The competition experiments were performed in the presence of *N. winogradskyi* in order to prevent the possible toxic effects of nitrite on the competing organisms. Each soil column contained 250 g of the moist soil described above and had an effective length of 15 cm. The construction and γ-sterilization of the soil percolation columns, the compositions of the media, and the performance of the experiment have been described previously (28). The inoculum contained 6.9×10^6 ammonium-oxidizing, 4.8×10^6 nitrite-oxidizing, and 1.2×10^7 heterotrophic bacteria per ml (fluorescent-antibody [FA] enumerations; see below), and its pH was 7.2. Percolation of the soil columns with mineral medium containing 5 mM ammonium was continued until 5 mM nitrate was found in the percolates. Then, the soil columns were inoculated with 10 ml of a log-phase culture of *A. peritocrescens* containing 5.4×10^5 flagellates per ml (counting chamber enumerations). New medium vessels containing the mineral medium described above supplemented with 0.40, 0.79, 1.19, 1.59, 1.98, or 2.38 g of glucose · H_2O per liter were connected. The glucose concentration in the medium for a soil column remained constant during the percolation period. Two columns were used for each glucose concentration, and four columns received no glucose.

Samples of the percolates were taken three times a week to determine ammonium, nitrite, nitrate, and glucose concentrations and pH. After 13 weeks, the soil columns were harvested. At the time of harvesting of the soil columns, dissolved oxygen concentrations were measured in every 0.5 mm of the uppermost 4 cm and at a depth of 7 cm of a soil column, which was percolated for 13 weeks with medium containing 5 mM ammonium and 10 mM glucose. Each column was divided into the following three parts: layer A, 0 to 3 cm; layer B, 3 to 6 cm; layer R, 6 to 15 cm. In each layer, the numbers of nitrifying bacteria were determined by the MPN technique. In addition, the potential ammonium- and nitrite-oxidizing activities were determined. The numbers of heterotrophic bacteria were determined by the FA enumeration technique, and those of flagellates were determined by the MPN technique. Soil mineral nitrogen concentrations and pH were determined in 1 M KCl and water extracts, respectively. The soil organic matter contents were also determined. Samples of the medium reservoirs were taken to determine ammonium and glucose concentrations and pH.

MPN enumerations. MPN enumerations were done as described previously (10, 23). The preparation of the soil suspensions, the dilution procedure, and the composition of the media for the enumeration of nitrifying bacteria have been described previously (28). Twelve replicates were made per dilution. Tests for ammonium and nitrite oxidation at the end of the incubation period (9 weeks) were performed with 0.04% (wt/vol) bromocresol purple (pH indicator) and the Griess Ilosvay reagents, respectively. Two suspensions per soil layer were made, and the suspensions were counted separately. To determine the number of flagellates, *A. globiformis* was used as the food bacterium in the incubations. A subsample (0.25 ml) of a soil suspension, 1:10 in 1 mM phosphate buffer (pH 7.0), was diluted fourfold in sterile microtiter plates (Costar, Cambridge, United Kingdom) containing 0.75 ml of a culture of *A. globiformis* (pH 7.5) per well. Twelve replicates were made per dilution. Plates were incubated at 20°C for 12 days. Tests for flagellate growth were done by using an inverted microscope. Two suspensions per soil layer were made, and the suspensions were counted separately. The MPNs of nitrifying bacteria and flagellates were obtained from statistical tables generated by a computer program (19).

FA enumerations. FA enumerations were done as described previously (25, 31). The extraction of the bacteria from the soil and the staining procedure on black polycarbonate membrane filters (pore size, 0.2 μm; Nuclepore Corp., Pleasanton, Calif.) (15) with antiserum prepared from blood from an immunized rabbit have been described before (28).

Determination of the potential nitrifying activities. Potential nitrifying activities were determined as described previously (26). The medium for the determinations contained the following (per liter): $(NH_4)_2SO_4$, 0.33 g; K_2HPO_4, 139 mg; and KH_2PO_4, 27 mg. The medium pH was 7.5. No inhibitors were applied, because sodium chlorate was also found to partially inhibit ammonium oxidation, whereas N-serve (nitrapyrin, 2-chloro-6-trichloromethylpyridine) did not completely inhibit ammonium oxidation (4). Samples (10.0 g) of well-mixed soil were mixed with 50 mg of $CaCO_3$ and 25 ml of the medium described above. The mixtures were incubated at 25°C and stirred at 150 rpm. The formation of nitrite and nitrate was followed for 6 h. Samples (1 ml) were taken every hour to determine the nitrite and nitrate concentrations. After centrifuging a sample at 15,000 $\times$ *g* in a Biofuge A table centrifuge for 5 min, 0.50 ml of the clear supernatant was mixed with 0.50 ml of a 2 M KCl solution. Samples were stored at 4°C and were analyzed within 1 day. The potential nitrifying activities were determined in duplicate. Potential ammonium-oxidizing activities were calculated from the sum of the amounts of nitrite and nitrate formed. The rates of nitrite and nitrate formation were calculated by linear regression. Potential nitrite-oxidizing activities could be calculated from the formation of nitrate when accumulation of nitrite appeared.

Determinations of mineral nitrogen concentrations, pH, and organic matter contents. Mineral nitrogen concentrations were determined by stirring 2.5 g of moist soil with 25 ml of a 1 M KCl solution. After 4 h, samples were taken and centrifuged at 15,000 $\times$ *g* in a Biofuge A table centrifuge for 5 min. The supernatant was stored at 4°C and was analyzed for mineral nitrogen compounds within 1 day. The pH-H_2O was determined by stirring 2.5 g of moist soil with 12.5 ml of demineralized water for 2 h. The organic matter contents were determined by the analysis of weight losses after heating samples of 5.00 g of dry soil at 550°C for 4 h. All determinations were done in duplicate.

Analytical methods. The concentrations of ammonium, nitrite, and nitrate were determined by using a Technicon Traacs 800 autoanalyzer (Technicon Instruments Corp., Tarrytown, N.Y.), which had a detection level of 0.01 mM N

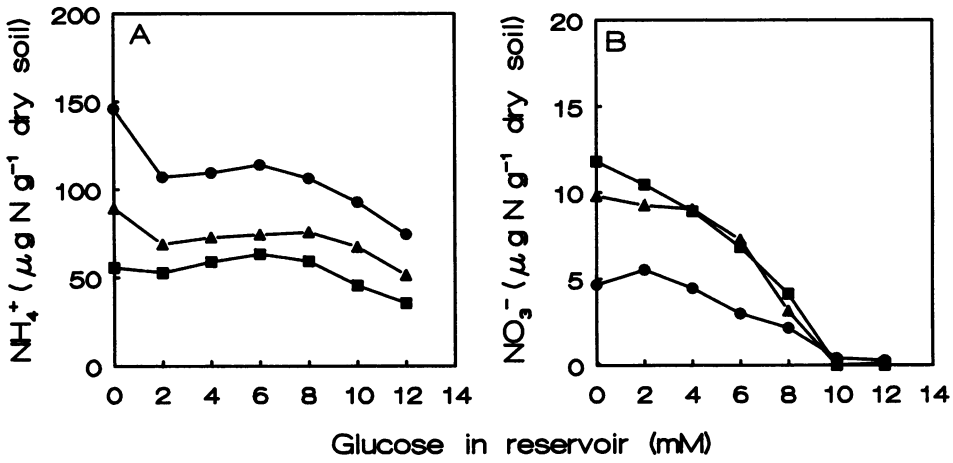

FIG. 1. Concentrations of ammonium (A) and nitrate (B) in 1 M KCl extracts (1:5) of the three layers of the soil columns containing *N. europaea*, *N. winogradskyi*, *A. globiformis*, and the flagellate *A. peritocrescens* after percolation for 13 weeks with media containing 5 mM ammonium and different concentrations of glucose at a dilution rate of 0.007 h^{-1} (liquid volumes) and 20°C. ●, layer A; ▲, layer B; ■, layer R. Data represent the means of duplicate soil columns. Duplicate values were within 10% of each other at all glucose concentrations.

for all three compounds. Glucose concentrations were determined with a test combination for glucose (Boehringer Mannheim Diagnostica, Mannheim, Germany) based on the photometric determination of glucose with glucose oxidase and peroxidase. The detection level for glucose was 0.05 mM. Dissolved oxygen concentrations were measured with an oxygen monitor (Strathkelvin Instruments, Glasgow, England) fitted with a microelectrode.

RESULTS

Nitrogen concentrations in mineral medium. In the soil columns percolated with mineral medium for 13 weeks, the 5 mM ammonium was completely converted into 5 mM nitrate by the nitrifying community. However, nitrate concentrations were lower at higher glucose concentrations, and they were zero at 10 and 12 mM glucose. The ammonium and nitrite concentrations in the percolates were below the detection level (0.01 mM N) at all glucose concentrations supplied. Nitrogen concentrations in the mineral medium percolates were comparable to those observed in a similar soil column experiment but in the absence of flagellates (28).

Ammonium concentrations in soil extracts decreased with increasing depth at all glucose concentrations supplied (Fig. 1A). When glucose was supplied up to 8 mM, the only considerable decrease in the ammonium concentration was observed in the upper layer, between 0 and 2 mM glucose. Above 8 mM glucose, the ammonium concentrations decreased slightly in all layers. Nitrate concentrations were lower in layer A than in layers B and R (Fig. 1B). They decreased in all three layers when the glucose concentration increased, and when the glucose concentration was greater than 10 mM, the nitrate concentrations were zero in all three layers.

Glucose concentrations. The glucose concentrations in the percolates were below the detection level (0.05 mM) during percolation at all glucose concentrations supplied. Glucose concentrations in extracts of the soil layers were not measured.

Numbers and potential ammonium-oxidizing activities of *N. europaea*. The MPNs of ammonium-oxidizing bacteria decreased as the glucose concentration increased in all three layers (Fig. 2A). The numbers of *N. europaea* also decreased with increasing depth at all glucose concentrations supplied. At 12 mM glucose, the numbers in layers A, B, and R amounted to 8, 7, and 9% of those found at 0 mM glucose, respectively.

The potential ammonium-oxidizing activities of *N. europaea* in the three layers decreased when the glucose concentration increased (Fig. 2B). In agreement with the numbers, the potential activities of *N. europaea* also decreased with increasing depth for all soil columns. At 12 mM glucose, the potential activities in layers A, B, and R amounted to 10, 16, and 11% of those observed at 0 mM glucose, respectively.

Numbers and potential nitrite-oxidizing activities of *N. winogradskyi*. The MPNs of nitrite-oxidizing bacteria decreased as the glucose concentration increased in all three layers (Fig. 3A). The numbers of *N. winogradskyi* also decreased with increasing depth, except for layers A and B at glucose concentrations greater than 8 mM, when the numbers were equal. At 12 mM glucose, the numbers in layers A, B, and R amounted to 7, 28, and 27% of those found at 0 mM glucose, respectively.

The potential nitrite-oxidizing activities decreased as the glucose concentration increased in all three layers (Fig. 3B). The potential activities of *N. winogradskyi* also decreased with increasing depth, in agreement with the numbers of *N. winogradskyi* and the potential ammonium-oxidizing activities. At 12 mM glucose, the potential activities in layers A, B, and R amounted to 11, 23, and 10% of those observed at 0 mM glucose, respectively.

Numbers of *A. globiformis*. The numbers of heterotrophic bacteria determined by FA enumeration in layer A were higher than those in layers B and R, which were equal, for all glucose concentrations supplied (Fig. 4). In layer A, the numbers of heterotrophic bacteria increased up to 6 mM glucose. At that concentration, the number was 17 times greater than the one observed at 0 mM glucose. For an unknown reason, the numbers of *A. globiformis* decreased above 6 mM glucose. In layers B and R, the numbers of *A. globiformis* increased up to 10 mM glucose. At that concentration, the numbers were 16 and 41 times greater than those found at 0 mM glucose, respectively.

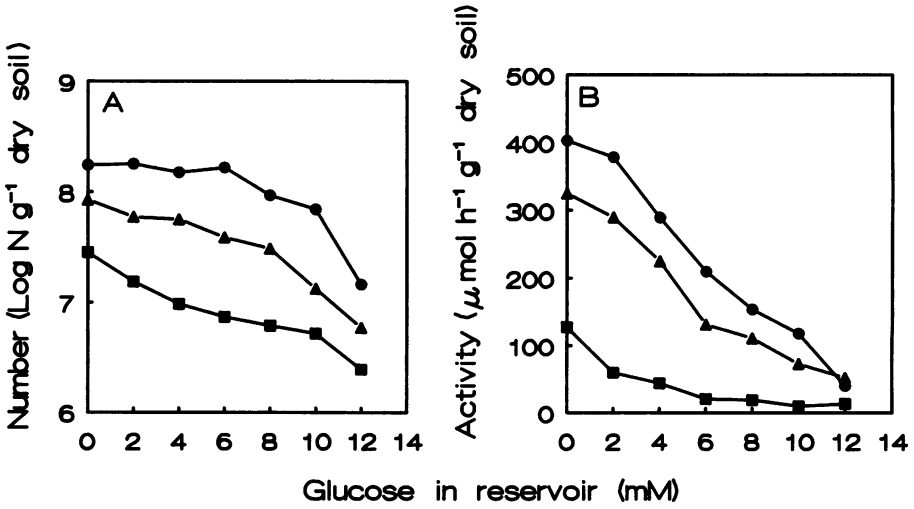

FIG. 2. Numbers (A) and potential ammonium-oxidizing activities (B) of *N. europaea* cells grown in the presence of *N. winogradskyi*, *A. globiformis*, and the flagellate *A. peritocrescens* in the three layers of the soil columns after percolation for 13 weeks with media containing 5 mM ammonium and different concentrations of glucose at a dilution rate of 0.007 h^{-1} (liquid volumes) and 20°C. ●, layer A; ▲, layer B; ■, layer R. Cells were enumerated by the MPN technique. Data represent the means of duplicate soil columns. Duplicate values were within 10% of each other at all glucose concentrations.

Numbers of *A. peritocrescens*. In layer A, the MPNs of flagellates increased as the glucose concentration increased until, at 8 mM glucose, a maximum of 1.4×10^5 organisms per g of dry soil was reached (Fig. 5). The numbers of flagellates found at concentrations of 8 to 12 mM glucose were 75 times greater than the ones found at 0 mM. In layer B, numbers of *A. peritocrescens* increased up to 12 mM glucose. At that concentration, the number was 33 times greater than the one observed at 0 mM glucose. In layer R, the numbers increased sharply above 8 mM glucose, and at 12 mM glucose, the number was 145 times greater than the one found at 0 mM glucose.

pH and organic matter contents. The mean pH in the percolates of the soil columns percolated with mineral medium was 7.3 (data not shown). The pH of the percolates increased linearly as the glucose concentration increased to 8.1 at 12 mM glucose. In the soil suspensions, the pH values in layer A were lower than those in layers B and R at all glucose concentrations supplied (data not shown). In the upper layer, the pH was 6.9 up to 4 mM glucose, but decreased to 6.4 between 4 and 12 mM glucose. The pH in the soil suspensions of layers B and R was 7.2 at all glucose concentrations supplied.

Soil organic matter contents in the columns percolated

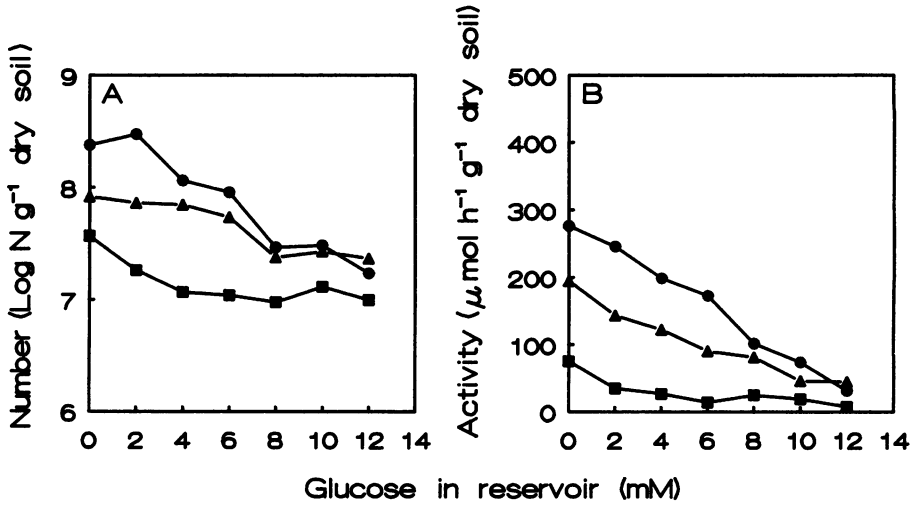

FIG. 3. Numbers (A) and potential nitrite-oxidizing activities (B) of *N. winogradskyi* cells grown in the presence of *N. europaea*, *A. globiformis*, and the flagellate *A. peritocrescens* in the three layers of the soil columns after percolation for 13 weeks with media containing 5 mM ammonium and different concentrations of glucose at a dilution rate of 0.007 h^{-1} (liquid volumes) and 20°C. ●, layer A; ▲, layer B; ■, layer R. Cells were enumerated by the MPN technique. Data represent the means of duplicate soil columns. Duplicate values were within 10% of each other at all glucose concentrations.

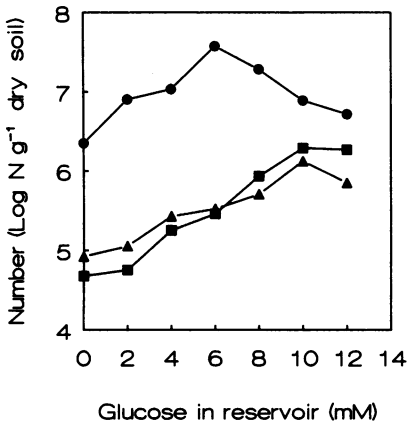

FIG. 4. Numbers of *A. globiformis* cells grown in the presence of *N. europaea*, *N. winogradskyi*, and the flagellate *A. peritocrescens* in the three layers of the soil columns after percolation for 13 weeks with media containing 5 mM ammonium and different concentrations of glucose at a dilution rate of 0.007 h^{-1} (liquid volumes) and 20°C. ●, layer A; ▲, layer B; ■, layer R. Cells were enumerated by the FA technique. Data represent the means of duplicate soil columns. Duplicate values were within 10% of each other at all glucose concentrations.

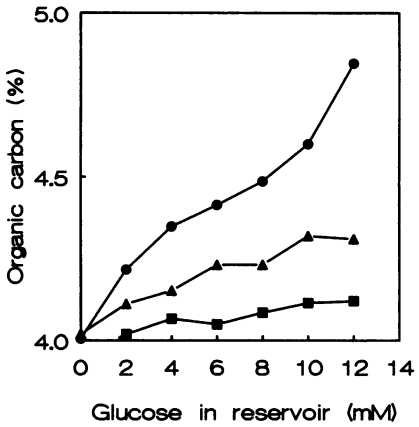

FIG. 6. Organic carbon contents in the three layers of the soil columns containing *N. europaea*, *N. winogradskyi*, *A. globiformis*, and the flagellate *A. peritocrescens* after percolation for 13 weeks with media containing 5 mM ammonium and different concentrations of glucose at a dilution rate of 0.007 h^{-1} (liquid volumes) and 20°C. ●, layer A; ▲, layer B; ■, layer R. Data represent the means of duplicate soil columns. Duplicate values were within 10% of each other at all glucose concentrations supplied.

with mineral medium were 4.0% for all three layers (Fig. 6). The organic matter contents decreased with depth for all glucose concentrations supplied. In layer A, the organic matter content increased as the glucose concentration increased and was 4.8% at 12 mM glucose. The largest increase in this layer was observed between 10 and 12 mM glucose, probably because of the presence of organic material excreted by the heterotrophic bacteria or the presence of glucose that had not been taken up. In layers B and R, the organic matter contents increased almost linearly as the glucose concentration in the reservoir increased.

Dissolved oxygen concentrations. The dissolved oxygen concentrations in the uppermost 2 cm of the soil columns percolated with medium containing 5 mM ammonium and 10 mM glucose decreased linearly from 95% to 50 to 60% of air saturation (data not shown). The dissolved oxygen concentrations between 2 and 4 cm and at 7 cm from the top of the soil columns were constant at 50 to 60% of air saturation. Therefore, it was assumed that below a depth of 2 cm, the dissolved oxygen concentration was constant at 50 to 60% of air saturation.

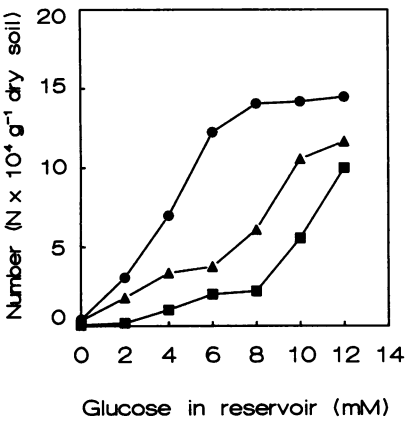

FIG. 5. Numbers of *A. peritocrescens* cells grown on *N. europaea*, *N. winogradskyi*, and *A. globiformis* in the three layers of the soil columns after percolation for 13 weeks with media containing 5 mM ammonium and different concentrations of glucose at a dilution rate of 0.007 h^{-1} (liquid volumes) and 20°C. ●, layer A; ▲, layer B; ■, layer R. Cells were enumerated by the MPN technique. Data represent the means of duplicate soil columns. Duplicate values were within 10% of each other at all glucose concentrations.

DISCUSSION

The presence of flagellates had different effects on the fate of ammonium in the soil columns at low and high glucose concentrations. At low glucose concentrations, at which the heterotrophic bacteria were limited by carbon, the nitrifying bacteria possibly oxidized ammonium more completely by the N-mineralizing activities of the flagellates, and consequently, the nitrate concentrations were slightly higher in the presence of flagellates than in their absence (28). However, the differences in nitrate concentrations were probably not significant. At high glucose concentrations, however, the N-limited heterotrophic bacteria immobilized all the attainable ammonium, including the part mineralized by the protozoa. In general, nitrate production is expected to be stimulated by grazing activities only in carbon-limited situations, whereas in nitrogen-limited situations, when nitrate production is generally low or absent, no direct effect of grazing on the production of nitrate can be expected.

The presence of flagellates in the soil columns apparently had no effect on the ultimate outcome of the competition for ammonium between the nitrification and the immobilization processes. In the presence as well as in the absence of flagellates, the heterotrophic *A. globiformis* won the competition for the available ammonium. However, the grazing activities of the flagellates in the present study had two marked effects on the fate of ammonium in the soil columns. The first one is related to the distribution of ammonium over the profiles of the soil columns (Table 1). In the absence as well as in the presence of flagellates, ammonium was found in KCl extracts of all soil columns after 10 or 13 weeks of

TABLE 1. Ammonium concentrations in the three layers of the soil columns containing *N. europaea*, *N. winogradskyi*, and *A. globiformis* in the absence and presence of the flagellate *A. peritocrescens*[a]

Glucose concn (mM) in reservoir	Ammonium concn (μg of N g^{-1} of dry soil)					
	Layer A		Layer B		Layer R	
	−F	+F	−F	+F	−F	+F
0	171	146	46	89	26	56
2	142	107	12	69	8	53
4	194	110	6	73	12	59
6	218	114	11	74	22	63
8	219	106	70	76	29	59
10	230	93	41	67	17	46
12	228	75	4	52	5	36

[a] Ammonium concentrations were determined after percolation for 13 weeks with media containing 5 mM ammonium and different concentrations of glucose at a dilution rate of 0.007 h^{-1} (liquid volumes) and 20°C. −F, absence of flagellate (28); +F, presence of flagellate.

TABLE 2. Specific activities of *N. europaea* grown in the presence of *N. winogradskyi* and *A. globiformis* and in the absence and presence of the flagellate *A. peritocrescens*[a]

Glucose concn (mM) in reservoir	Sp act (pmol of NH_4^+/cell/h)					
	Layer A		Layer B		Layer R	
	−F	+F	−F	+F	−F	+F
0	0.30	2.31	0.63	3.83	1.39	4.47
2	0.43	2.11	0.73	4.86	1.43	3.88
4	0.28	1.93	0.51	3.97	1.37	4.55
6	0.18	1.27	0.37	3.38	1.32	2.80
8	0.06	1.65	0.25	3.61	1.44	3.10
10	0.01	1.70	0.28	5.49	1.02	1.89
12	0	2.76	0.60	8.87	1.24	5.47

[a] Cells were enumerated by the MPN technique, and activities were determined by potential ammonium-oxidizing activity measurements in the three layers of the soil columns after percolation for 13 weeks with media containing 5 mM ammonium and different concentrations of glucose at a dilution rate of 0.007 h^{-1} (liquid volumes) and 20°C. −F, absence of flagellate (28); +F, presence of flagellate.

percolation. In the absence of flagellates, relatively high amounts of ammonium accumulated in the upper layer (0 to 3 cm), whereas in the underlying layers the ammonium concentrations were low (28). We assume that this KCl-extractable ammonium was not available for or attainable to the heterotrophic and nitrifying bacteria. It was suggested that the relatively high ammonium concentrations were due to a local separation of the ammonium and the bacteria, which were possibly growing in microcolonies. Incomplete nitrification in a continuous-flow nitrification column has previously been reported by Cox et al. (9). In the presence of flagellates, the distribution of ammonium over the profiles was more uniform than in their absence. The ammonium concentrations decreased with increasing depth, and in layers B and R considerable amounts of ammonium were found, whereas less ammonium accumulated in the upper layer (Table 1). The mechanism leading to a more even distribution of ammonium in the presence of flagellates is not clear. Possibly, transportation of medium containing ammonium toward microcolonies of nitrifying bacteria, which are more or less fixed at regular places in the soil, occurred by the movement of the flagellates.

A more even distribution of ammonium or nitrifying bacteria over the soil column may have led to the second prominent effect of the flagellates, which was related to the potential ammonium-oxidizing activities of *N. europaea* in the layers of the soil columns (Table 2). The numbers of ammonium-oxidizing bacteria were comparable in the presence and absence of flagellates. Also, the decreases in the numbers of ammonium-oxidizing bacteria with increasing glucose concentrations were comparable, since in the presence of flagellates the MPNs of *N. europaea* at 12 mM glucose were about 10% of those at 0 mM glucose, whereas in the absence of flagellates the MPNs at 12 mM glucose in layers A, B, and R amounted to 15, 14, and 27% of those at 0 mM glucose, respectively (28). In spite of this, the potential ammonium-oxidizing activities were four to five times higher in the presence of flagellates than in their absence. At all glucose concentrations supplied and in all three soil layers, the specific activities of *N. europaea* cells were higher in the presence of flagellates than in their absence. In spite of the predation pressure, the activity of the nitrifying bacteria was apparently stimulated in the presence of protozoa. Stimulation of the activity of nitrifying bacteria in the presence of bacteriophagous protozoa was reported before by Griffiths (14), who found that the higher nitrifying activity in the presence of protozoa was not due to the extra amount of mineralized ammonium available to the nitrifying community; he suggested that it was due to an unknown growth factor excreted by the flagellates. The stimulation of nitrification in the more heterogeneous soil could also be explained by an activity of the flagellates which increases the availability of that part of the ammonium, which is inaccessible to the nitrifying bacteria in the absence of protozoa. The transportation of medium containing ammonium toward the nitrifying bacteria mentioned above might have played a role in this.

As mentioned above, the heterotrophic species *A. globiformis* won the competition for the available ammonium, in agreement with similar soil column experiments without flagellates (28). This was concluded from the strong decreases in the potential ammonium-oxidizing activities (Fig. 2B). At 12 mM glucose, the potential activities of *N. europaea* in the different soil layers decreased to 10 to 16% of those found at 0 mM glucose. This indicates that the heterotrophic bacteria almost completely outcompeted the nitrifying bacteria at high glucose concentrations. The competition for ammonium between nitrifying and heterotrophic bacteria in soil columns takes place as long as there is a buildup of a heterotrophic population during the percolation period (29). Also, in other studies heterotrophic bacteria were found to be more competitive than nitrifying bacteria for ammonium (17, 21, 22, 29). An alternative explanation for the results presented here is that the nitrification process was inhibited by glucose or by organic compounds originating from the heterotrophic bacteria or flagellates. However, these inhibitory effects can be excluded here, since in separate experiments, it was found that neither glucose nor the supernatant of a culture of *A. globiformis* containing products of glucose metabolism affected the ammonium oxidation rate by *N. europaea* (29). The addition of a supernatant of a culture of flagellates to a batch culture of actively nitrifying bacteria, which contained a surplus of ammonium, was found not to affect the nitrification rate.

As mentioned above, comparable numbers of ammonium-oxidizing bacteria were found in the soil columns in the presence and absence of flagellates at all glucose concentrations supplied. These results do not agree with those from

similar competition experiments in continuous cultures, in which the numbers of *N. europaea* in the presence of flagellates were, at most, only 10% of those in the absence of protozoa at all glucose concentrations supplied. The difference between the two model systems may be explained by assuming that the growth of nitrifying bacteria appeared in so-called microniches, as was already suggested above. A part of these microniches might have been inaccessible to the flagellates, giving the nitrifying bacteria protection against predation. The role of microniches in protecting bacteria against predation in soil has previously been demonstrated by Postma et al. (20).

The numbers of flagellates corresponded well to the numbers of heterotrophic bacteria, although the majority of cells in the soil were nitrifying bacteria. For example, the ratios between the numbers of the two nitrifying species and *A. globiformis* at 2 mM glucose in layer A amounted to 231:1 in the absence of flagellates and 22:1 in their presence. This difference in ratio was due to the numbers of heterotrophic bacteria, which in the presence of protozoa were one order of magnitude higher than those in their absence, whereas the numbers of nitrifying bacteria were more or less equal at similar glucose concentrations. Although heterotrophic cells were added to the soil columns together with the inoculum of flagellates, a reason for the higher numbers of *A. globiformis* in the presence of protozoa cannot be given. Increases in bacterial numbers in the presence of bacteriophagous protozoa have been reported before (5). This result does not agree with the one obtained in similar competition experiments in continuous cultures, in which the numbers of heterotrophic and nitrifying bacteria in the presence of protozoa were one order of magnitude lower than those in their absence (29, 30). An explanation for this difference between the two model systems cannot be given. Possibly, growth in microniches, which were partly inaccessible to the protozoa, may again have given some protection against predation, or the excretion of an unknown growth factor by the protozoa may have stimulated growth of the heterotrophic bacteria. There was no further increase in the numbers of flagellates in the presence of 8 mM glucose or greater, which is in agreement with the numbers of heterotrophic bacteria. Apparently, the protozoa did not reduce the bacterial numbers below a certain level. Again, protection from predation by growth in inaccessible microniches might have played a role in this. This cannot be the only explanation, because Sambanis and Fredrickson (24) found that in batch as well as in homogeneous continuous cultures, 10^4 to 10^7 viable bacteria persisted in the presence of actively growing protozoa. Insufficient numbers of ingested bacteria restrict the growth of the flagellates.

The experiment in soil columns gives insight into the basic relationships between nitrifying bacteria, heterotrophic bacteria, and protozoa. *A. globiformis* was again more competitive than *N. europaea* for limiting amounts of available ammonium. At low glucose concentrations, the nitrifying bacteria benefit from the nitrogen mineralized by the flagellates, and consequently, small increases in the nitrate concentrations of the percolates were observed. Such experiments are indispensable to understanding the relationships between the functional groups in more complicated systems, such as natural soils covered by vegetation.

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