6.7. POPULATION DYNAMICS AND NITROGEN MINERALIZATION RATES IN SOIL AS INFLUENCED BY BACTERIAL GRAZING NEMATODES AND MITES [1]

L. Brussaard, J.P. Kools, L.A. Bouwman and P.C. de Ruiter

Institute for Soil Fertility, P.O. Box 30003, 9750 RA Haren, The Netherlands

INTRODUCTION

The work presented in this paper is part of the Dutch Programme on Soil Ecology of Arable Farming Systems (Brussaard *et al.*, 1988). This programme includes a comparison of the functioning of the soil/plant ecosystems of high and reduced input arable farming at the Lovinkhoeve experimental farm. This farm is located on a calcareous sandy loam soil in the Noordoostpolder, which was reclaimed from Lake Yssel in 1942. In our case reduced input means a smaller nitrogen addition and a larger proportion of nitrogen added as manure. The use of organic manure might contribute to a solution for the great manure surpluses in the Netherlands. In organic-nutrient, input farming the crop is more dependent on the activity of soil organisms for its supply with nutrients. Appropriate management of the soil biota may help to improve the efficiency of nutrient use in agroecosystems (Elliott and Coleman, 1988). Therefore, knowledge of the relation between population dynamics in the soil ecosystem and the rate of nitrogen mineralization is required.

We carried out a microcosm experiment in which we studied the population dynamics of bacteria, protozoa, nematodes and mites and the effects of these organisms on nitrogen mineralization. The experiment comprised four series: bacteria with protozoa, bacteria with protozoa and nematodes, bacteria with protozoa and mites and bacteria with protozoa, nematodes and mites, We used the nematode *Acrobeloides bütschli* and the mite *Histiostoma litorale*, both of which are assumed to feed on bacteria only. So we had possible competition for bacteria among amoebae, flagellates, nematodes and mites. Protozoa and nematodes, both separately and in combination, have been shown to affect the population dynamics of bacteria and to stimulate the nitrogen mineralization in microcosms (Clarholm, 1981; Coleman *et al.*, 1977; Coleman *et al.*, 1983). Mites and springtails generally accelerate the decomposition of litter but they stimulate the mineralization of nitrogen in only half of the cases reviewed by Seastedt (1984). Predatory mites may reduce the number of

---

bacteriovorous nematodes so that the latter do not overgraze the microflora to such an extent that the mineralization of nitrogen is retarded (Santos *et al.*, 1981; Elkins and Whitford, 1982) but this effect is not always apparent (Parker *et al.*, 1984). However, we are not aware of any studies concerning bacteriovorous mites.

The objective of the present study was to assess the influence of possible competitors at the grazer's trophic level on the numbers of bacteria as well as bacterial grazers and on the concomitant nitrogen mineralization.

MATERIAL AND METHODS

We filled 72 glass pots of 1 l, four series of 18 pots each, with 600 g (dry weight) of a calcareous sandy loam soil (pH-KCL 7.3; organic matter content 2.4%; mineral nitrogen content 12 mg/kg), to which 1 g lucerne meal was added, i.e., 60 mg N per kg soil. The soil was sterilized by γ-radiation (2.5 MRad) after drying at 5°C to 30% WHC and passed twice through a 8 mm sieve.

One series of pots was inoculated with a soil suspension with bacteria and protozoa (amoebae and flagellates); this was the BP-treatment. Bacteria and protozoa were collected by filtering a suspension of the soil through a 25 μm filter. The soil from which the suspension was made came from the same site as that added to the pots and had been heat-treated (24 hours at 50°C, two days at room temperature, 12 hours at 50°C) to kill nematode eggs that might have passed the filter. The second series received this suspension plus the nematode species *Acrobeloides bätschli* (BPN-treatment). *Acrobeloides bätschli* was cultured in soil (heat-treated as described above), to which lucerne meal and a non-heat-treated soil suspension, filtered through a 1.5 μm filter, had been added. The nematodes were eluted with sterile water and added in suspension to the pots (approximately 2000 per pot, i.e. approximately 3 per g soil). The third series received the heat-treated suspension plus the mite species *Histiostoma litorale* (BPH) and the fourth the suspension plus nematodes and mites (BPNH). *Histiostoma litorale* is an anoetid mite measuring 400-500 μm in length (deutonymph to adult). It has chelicerae transformed to combs that cause a water current from which bacteria are filtered under moist conditions (Woodring, 1963). The mites were cultured on agar plates (15% agar + some glycine) and 25 individuals were added per pot, i.e., approximately 4 per 100 g soil. Both *A. bütschli* and *H. litorale* are bacteriovorous and among the most abundant species captured in the soil of our experimental fields. The moisture content of the soil in the pots was adjusted to 24% (w/w). The bulk density was 1.39×10^3 kg m^{-3}.

The pots were incubated at a light/dark and temperature regime comparable with that in the field in spring (L/D:14/10); the temperature was set at 14°C during the day and 9°C during the night. We found that under these conditions the life cycle of the mite is 2-2.5 weeks. The pots of each series were analysed after 1, 2, 3, 6, 9 and 14 weeks. At each date the number of bacteria, amoebae, flagellates, nematodes and mites and the amount of mineral nitrogen were measured in three replicates.

Bacteria were counted by the plate dilution method (medium 1.5% agar, 0.1% glucose, 0.1% proteose peptone (Prescott and James, 1955). Protozoa were counted by the most-probable-number method (same medium plus *Pseudomonas fluorescens* as food) (Darbyshire *et al.*, 1974). Nematodes were collected by the elutriation method and then counted (Oostenbrink, 1960). Mites were collected by high-gradient extraction and then counted. Mineral nitrogen was extracted with 1N KCL and determined colorimetrically.

The numbers of organisms were approximately lognormally distributed, so analyses of variance were applied to the log-transformed data.

RESULTS

The numbers of the organisms in the four different treatments BP, BPN, BPH and BPNH are presented in Fig. 1 and a summary of the analyses of variance is given in Table 1. Bacterial numbers were approximately the same in all treatments (Fig 1a, Table 1).

Larger numbers of amoebae were found when mites were present ($p < 0.001$) (Fig. 1b). There was an interaction between the effect of nematodes and that of mites on amoebae ($p < 0.001$): the number in the BPNH treatment was lower than in the BPH treatment, whereas the number in the BPN treatment exceeded the number in the BP treatment (Fig. 1b). Comparison of the BPH and the BP treatments, shows that the presence of mites resulted in a 15-fold increase in numbers of amoebae.

Table 1. Effect of nematodes (nema) and mites (mite) on the numbers of bacteria (bact), amoebae (amoe), flagellates (flag), nematodes and mites and the amount of mineral N (Nmin)

	Significance		
	Nema	Mite	Nema and mite
Bact	ns	ns	ns
Amoe	ns	<.001	<.001
Flag	<.001	ns	<.05
Nema	-	<.01	-
Mite	ns	-	-
Nmin	<.001	ns	<.05

The number of flagellates was significantly larger in the nematode treatments ($p < 0.001$, Fig. 1c). This effect was mainly due to different numbers of flagellates at the beginning of the experiment: approximately 500 per g soil in the treatments without nematodes compared with 5.15^5 per g soil in the nematode treatments. The interaction between the effect of mites and that of nematodes ($p < 0.05$) consisted in a slight reduction in the effect of nematodes in the presence of mites.

Mites significantly reduced the number of nematodes ($p < 0.01$, Fig. 1d) by approximately 23%.
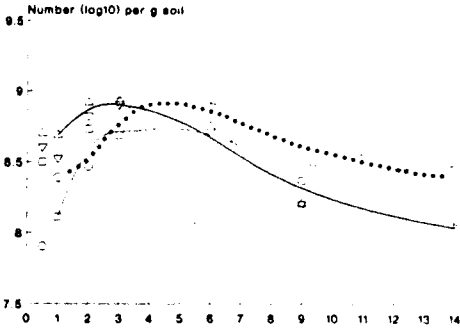
Mites were found in smaller numbers when nematodes were present (Fig. 1e), but this difference was not statistically significant.

Nematodes significantly increased ($P < 0.001$) the amount of mineral N by approximately 22%. The effect of mites depended on the presence of nematodes: an increase without nematodes (approximately 10%) and no effect when nematodes were present (Fig. 1f).
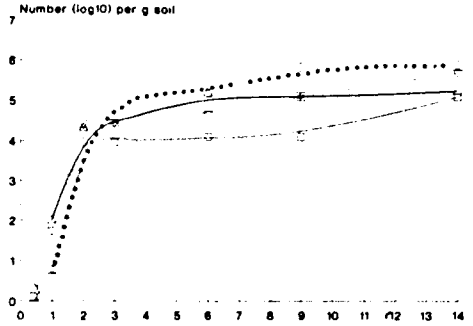
DISCUSSION

The results show that the system we studied probably consisted of more trophic interactions than we assumed before hand (Fig. 2). The stimulating effect of mites on the number of amoebae indicates that the feeding rate of the amoebae was increased by mites. The accessibility of bacteria to the amoebae may have been improved by *H. litorale* due to the feeding mode of the mite, which cause water currents around its chelicerae (Woodring,
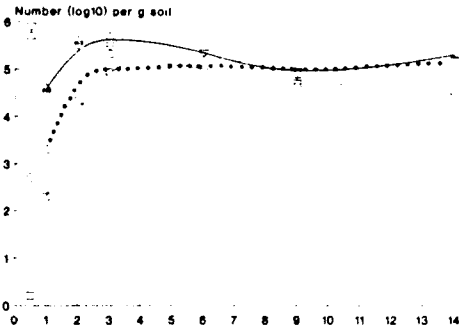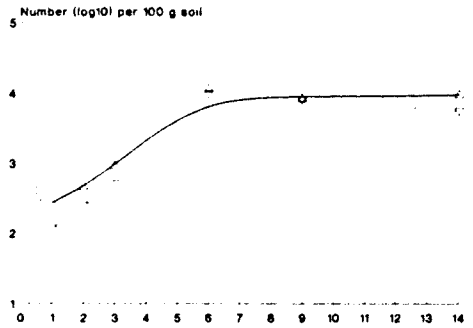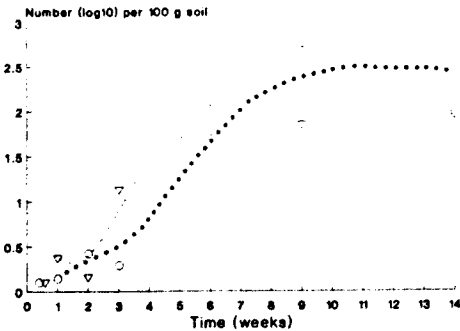
(a) bacteria

Number (log10) per g soil

(b) amoebae

Number (log10) per g soil

(c) flagellates

Number (log10) per g soil

(d) nematodes

Number (log10) per 100 g soil

(e) mites
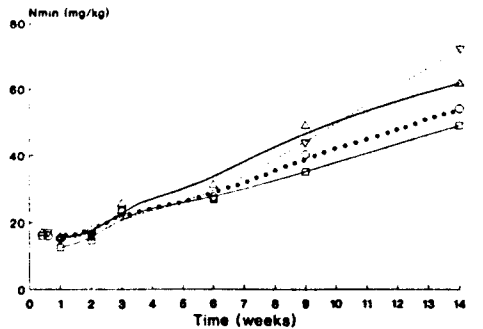
Number (log10) per 100 g soil

Time (weeks)

(f) mineral N

Nmin (mg/kg)

Time (weeks)

Fig. 1.   Effect of food-web composition on numbers of organisms.
a. Bacteria. b. Amoebae. c. Flagellates. d. Nematodes. e. Mites. f. Mineral nitrogen. Initial quantities
are given by the separate points.

□ : BP: bacteria + amoebae + flagellates
Δ : BPN: bacteria, amoebae, flagellates and nematodes
o : BPH: bacteria, amoebae, flagellates and mites
∇ : BPNH: bacteria, amoebae, flagellates, nematodes and mites.
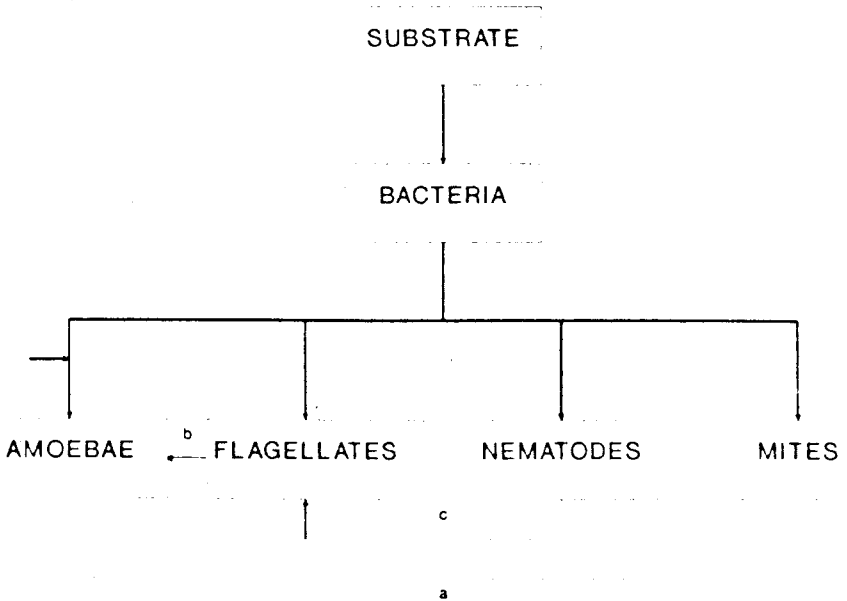Each symbol is the mean of three replicates.

Fig. 2. Conceptual model of the food web studied:
Broken line (a) denotes the stimulating effect of mites on consumption by amoebae; broken line (b) denotes the feeding on flagellates by amoebae; broken line (c) denotes that nematodes are accompanied by large numbers of flagellates.

1963). The feeding rate of the flagellates might also have been stimulated by the mites, but this fact was possibly obscured by feeding of amoebae on flagellates.

The presence of large initial numbers of protozoa, especially the flagellates, in the series with nematodes indicates that by introducing nematodes we also introduced large numbers of protozoa. We also observed that one species of protozoa was present in the treatments with nematodes only.

The reduction of the number of nematodes by mites might be the direct consequence of competition between nematodes and mites, but it might also be due to the relatively large numbers of amoebae in the mite treatments. Since mites consumed only a small proportion of the bacteria in comparison with the amoebae, we attribute the decrease in number of nematodes to the large number of amoebae.

Nematodes increased the number of amoebae in the series without mites, but decreased the number of amoebae in the series with mites. These results can be explained by assuming a two fold effect of nematodes. Firstly, flagellates may have served as food for the amoebae, so that by introducing nematodes accompanied by flagellates, nematodes stimulated the growth of amoebae. Secondly, the presence of namatodes and the large numbers of flagellates in the nematode treatments decreased the numbers of mites and thereby the positive effect of mites on the growth of the amoebae.

A positive effect of nematodes on nitrogen mineralization is in agreement with observations of Anderson et al. (1983) concerning nitrogen mineralization and Coleman et al. (1978) concerning soil respiration. In our case the relatively high mineralization rate in the nematodes treatment possibly had three causes: (I) the direct effect of the nematodes

feeding on bacteria, (II) the large numbers of flagellates accompanying the nematodes feeding on bacteria, and (III) the large numbers of flagellates fed upon by the amoebae. These flagellates feed on bacteria and are themselves fed upon by amoebae, which thereby add to the amount of nitrogen mineralized.

In summary, the effects of nematodes and mites on the number of amoebae and on nitrogen mineralization indicate that mites stimulate the feeding rate of the amoebae and that amoebae feed on flagellates. Whether these assumed interactions provide a suitable explanation of the experimental findings was examined by means of simulation. Three alternative hypotheses were compared. The first included all assumed interaction, i.e. amoebae, flagellates, nematodes and mites feeding on bacteria, nematodes "carrying" large numbers of flagellates, amoebae feeding also on flagellates and mites stimulating the amoebal feeding rate on bacteria. In the second, mites did not stimulate the feeding rate of the amoebae and in the third the amoebae did not feed on flagellates. The outcome of this simulation showed that only the first hypothesis could account for the observed effects of mites and nematodes on the number of amoebae and on the rate of nitrogen mineralization, whereas the other two hypotheses, irrespective of the parameter values used, did not generate comparable results as were observed in the experiment.

The results also show that after approximately four weeks all species had reached their maximum density, whereas mineralization took place mainly after the fourth week. This indicates that nitrogen (and carbon) are not mineralized immediately after feeding. Such a time interval between feeding and net mineralization has also been reported for aquatic systems with bacterial grazers (Bloem et al., 1988).

Finally we conclude that a relatively small "pool" of mites may cause a relatively large increase of the "flow" of organic material: the amount of organic carbon in the mites was approximately 1 µg per g soil, whereas the mites caused an increase in amoebal carbon of approximately 25 µg per g soil. The mites also significantly reduced the stimulating effect of the nematodes on the nitrogen mineralization rate. This indicates that in terms of both carbon and nitrogen transformations relatively small populations of faunal groups may have distinct effects on the functioning of complex ecosystems.

SUMMARY

A microcosm experiment was carried out in which we studied the influence of four bacterial grazers (amoebae, flagellates, nematodes and mites) on the numbers of bacteria, on the numbers of other grazers and on the concomitant nitrogen mineralization.

Mites increased the number of amoebae. This increase was reduced by nematodes. Nematodes increased the number of flagellates and the rate of nitrogen mineralization. Mites reduced the positive effect of nematodes on the mineralization rate.

The results led to the hypothesis that mites promote bacterial consumption by amoebae, that nematodes carry protozoa, and that amoebae feed on flagellates.

ACKNOWLEDGEMENTS

REFERENCES

Anderson, R.V., W.D. Gould, L.E. Woods, C. Cambardella, R.E. Ingham D.C. Coleman. 1983. *Oikos* **40**: 75-80.

Bloem, J., C. Albert, M.-J.B. Bär-Gilissen, T. Berman and T.E. Cappenberg. 1988. *Plankton Res.* (accepted for publication).

Brussaard, L., J.A. van Veen, M.J. Kooistra and G. Lebbink. 1988. *Ecological Bulletins* **39**: 35-40.

Clarholm, M. 1981. *Microbial Ecology* **7**: 343-350.

Coleman, D.C., R.V. Anderson, C.V., Cole, E.T. Elliott, L.E. Woods and M.K. Campion. 1978. *Microbial Ecology*, **4**: 373-380.

Coleman, D.C., C.V. Cole, R.V. Anderson, M. Blaha, M.K. Campion, M. Clarholm, E.T. Elliott, H.W. Hunt, B. Schaefer and J. Sinclair. 1977. *Ecological Bulletins*, **25**: 299-309.

Coleman, D.C., C.P.P. Reid and C.V. Cole. 1983. *Advances Ecol. Res.* **13**: 1-55.

Darbyshire, J.F., R.E. Wheatley, M.P. Greaves and R.H.E. Inkson. 1974. *Rev. Ecol. Biol. Sol.* **11**: 465-475.

Elkins, N.Z. and W.G. Whitford, 1982. *Oecologia*, **55**: 303-310.

Elliott, E.T. and D.C. Coleman. 1988. *Ecological Bulletins* **39**: 23-32.

Oostenbrink M. 1960. Estimating nematode populations by some selected methods. In: *Nematology*, J.N. Sasser and W.R. Jenkins (eds). University of North Carolina Press, Chapel Hill: 85-102.

Parker, L.W., P.F. Santos, J. Phillips and W.G. Whitford. 1984. *Ecological Monographs*, **54**: 339-360.

Prescott, D.M. and T.W. James. 1955. *Exp. Cell Res.* **8**: 256-258.

Santos, P.F., J. Phillips and W.G. Whitford, 1981. *Ecology*, **62**: 664-669.

Seastedt, T.R. 1984. *Ann. Rev. Entomol.* **29**: 25-46.

Woodring, J.P. 1963. In: *Advances in Acarology* vol 1, J.A. Naegele (ed.) Comstock Publ. Ass., N.Y.