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RELATIONSHIP BETWEEN SOIL TEXTURE, MINERALIZATION RATE OF NITROGEN, CARBON AND MICROORGANISMS IN SOIL

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ABSTRACT

Texture affects pore space, bacteria and protozoan populations and their activity in soil. The objective of this study was to test the hypothesis that protozoa grazing on bacteria increase the mineralization of bacterial C and N more in coarse-textured soils than in fine-texture soils. The microcosm experiment consisted of samples from three sterilized Silty clay (SIC); clay loam (CL) and silty (SL) soils inoculated with *pseudomonas* bacteria, two treatments (with and without protozoa), and five sampling dates. The *Pseudomonas* population was labelled in situ by adding glucose-^{14}C and KNO_3-^{15}N (day 0). A species of *Acanthamoeba* was added to the microcosms on 3rd day . On the 5th bacterial numbers in all three soils were approximately $4x10^8$ g^{-1} soil. The greatest reduction of bacteria due to protozoan grazing occurred between 5th and 8th days. All soils showed increased CO_2-^{14}C evolution and NH_4-^{15}N mineraliztion due to protozoan grazing but the mineralization rate of labelled N in the (SL) soil was much greater than in the fine-textured soils. The effect of texture apparent in the coarse-texture soils.on protozoan grazing was not as marked between 13th and 38th days as earlier in the incubation. Protozoan-induced effects were transient in the soils studied and were most

Keywords: Soil texture, ^{14}C, ^{15}N, N mineralization, imm obilizaiton, bacteria, organic matter, porosity, protozoa, bulk density, C:N ratio.

INTRODUCTION

In arable soils, free-living protozoa regulate the number of bacteria and influence nutrient cycling in the short term (Bamforth , 1985). Microcosm studies have shown that microbial turnover rates and mineralizaiton are often increased when microbial predators are present (Griffiths, 1986; and Kuikman and Van Veen ,1989). When fauna respire CO_2-C,N should be mineralized because the C:N ratio of fauna is similar to the bacteria that are grazed . Nitrogen mineralized by protozoa makes N more available to microbes and plants under N-limiting conditions and may be important in decreasing the limitation on plant growth or microbial decomposition rate of high C:N ratio substrates (Robinson *et al.* ,1989).

Turnover of C and N within the microbial biomass is faster in coarse-texture than in fine-textured soils .Textural effects may impose physical restrictions on the ability of fauna to graze on microbes ; therefore, texture may play a role in faunal-induce mineralization of microbial C and N. Elliott *et al.*, (1980) found, in part, that amoeba grazed bacteria more in a sandy loam subsoil than in a clay subsoil. This was characterized by higher amoebal

numbers and a greater increase in CO_2-C evolution in the sandy loam. The effect of texture on N mineralization was not reported. Studying the protozoa-induced mineralization of bacterial sulfur, Gupta and Germida (1989) found that amoeba in a loamy sand soil reduced bacterial numbers more than in a sandy clay loam soil. The rate of CO_2-C production in loamy sand increased more than in the sandy clay loam soil when protozoa were added. A higher number of large pores in the loamy sand soil could explain the larger grazing effect compared to the sandy clay loam soil.

Carbon and N mineralization during laboratory incubation, expressed as a proportion of microbial C or N, was greater in soil (Rutherford and Juma,1989a,b). Pratozoan numbers prior to incubation tended to be greater. It is possible that protozoa in the coarser-textured Luvisol grazed more bacteria than in the finer-textured, partly contributing to the different mineralization rates in the two soils. Few studies have reported the effects of soil texture on bacterial protozoan interactions in soil, including C and N mineralization. An integrated study is needed to measure the effects of texture on dynamics of bacteria, C and N mineralization in the presence and absence of predators.

The objective of this study was to test the hypothesis that protozoa grazing on bacteria increase the mineralization of bacterial C and N and that this effect would be more apparent in coarse-textured that fine-textured soils.

MATERIALS AND METHODS

The Soils:

Three soils Silty clay (SIC); Clay loam (CL) and Silty loam (SL) in cereal production for at least 10 years were sampled from the Assiut Governorate area, and the samples were stored at 4°C until use. Soil samples were taken from the Ap horizon (0-to 30-Cm depth) after cereal harvest but prior to disking of the residues. Soils were drying sieved (<2mm) field moist and then were dired to –1.5 Mpa water potential. Selected soil properties are presented in Table 1.

All soils had fine granular structure after sieving, but Soil C (SL) had a large number of non-aggregated sand particles within the matrix. Stereomicroscope examination showed that the sand grains in soil C were coated with fine minerals and organic matter, similar to those found in another coarse-textured from Assiut Valley. Subsamples of the three soils were adjusted to the bulk densities given in Table 1 prior to pressure plate determination of moisture retention . Effective pore diameter (d in μm) was predicted from the formula $d = 43\Psi_m$ where 2 is surface tension (7.27×10^{-2} Mpa μm at 20°C) and Ψ_m is suction (MPa) (Papendick and Campbell, 1981). Soil was weighed (100g moist soil) into 200-ml. autoclavable plastic cup assemblies. The cups were designed to allow gas exchange from soil at the top and bottom of each cup without microbial contamination. Cup assemblies with soil were sterilized by autoclaving (1h at 121°C and 140 K P_a) three time over a 6-day period. Matric potential of the three soils after autoclaving was approximately-1.0 MPa (~0.3 μm water-filled pore diameter).

General Experimental Design:

The experiment consisted of three soils, two treatments (with and without protozoa), and five major sampling dates. Soil A (SiC) and soil B(CL) treatments were replicated four times for each sampling date and Soil C (SL) treatments were replicated twice.

Inoculation of Bacteria Into Sterile Soils:

Preliminary work showed that a fluorescent *Pseudomonas* species was common to the three soils. This organism was isolated on 0.1 strength tryptone soya agar and then cultured for 2 days in Prescott-Jamco medium (PJM) with a mixture of micronutrients containing 0.2% peptone and 0.2% glucose (PH7) (Page, *et al.*,1982). The cultured bacteria averaged 0.75 μm in diameter and 2.2μm in length. The culture was centrifuged for 15 min at 7000xg and was washed once in PJM before inoculation into the sterilize soils using a syringe ($1x10^7$ $CFUg^{-1}$ oven dry soil). The soils were then gently mixed and packed to the appropriated bulk density (Table 1). Soil matric potential was raised to-0.2 MPa (~1.5 μm water-filled pore diameter) after bacterial inoculation.

Labelling of Bacterial Biomass:

The bacterial population was labelled in situ with ^{14}C and ^{15}N prior to protozoa inoculation. Glucose-^{14}C (specific activity = 13 K B_q mg^{-1} C) and KNO_3-^{15}N (excess= 4.55%) were added to the soils at rates of 36/μg $^{14}Cg^{-1}$ soil and 31 μg $15Ng^{-1}$ soil, respectively, 5 days after bacterial inoculation into sterile soils. The soils were then gently mixed and packed to the appropriate bulk densities (Table 1). The cup assemblies were placed into disinfected 1.2 L^{-1} glass jars containing a CO_2-C trap (20 ml. of 0.5 M NaOH). Each cup assembly-jar unit was referred to as a microcosm. Glass jars containing NaoH served as CO_2 blanks. Microcosms and CO_2 blanks were incubated in darkness at 22ºC. Soil matric potential was –0.05 MPa (~5.8 μm water-filled pore diameter) after C and N amendments. The day of glucose and KNO_3 addition was designated as day 0.

Although the soils contained NH_4-N and NO_3-N before glucose-^{14}C addition, KNO_3-^{15}N was added on day 0 to label the bacterial biomass and follow the N dynamics. Bacterial growth on glucose would cause immobilization of some of the KNO_3-^{15}N. Subsequent mineralization would result in the release of NH_4-^{15}N. Since nitrifiers were no present in the autoclaved soil, the NH_4-^{15}N would not be further transformed into NO_3-^{15}N. The accumulated NH_4-^{15}N would be due to bacterial and/or amoebal mineralization. Therefore, the dynamics of three ^{15}N pools were studied: (i) NO_3-^{15}N which remained in the soil and was not taken up by bacterial biomass; (ii) NH_4-^{15}N which originated from the bacterial biomass and was mineralized during bacterial turnover and protozoan grazing; and (iii) organic-^{15}N in soil that was mainly generated by the microbial biomass or its by-products.

Protozoa Inoculation :

Preliminary work showed that an *Acanthamoeba* species (Lousier and Bamforth ,1990) was common to the three soils. This small amoeba was approximately 10 μm in diameter when observed on a microscope slide, but it was able to pass through polycarbonate Nucleopore filters of 3μm diameter, but not of 2 μm diameter or smaller . The amoeba was isolated and then cultured for 6 days on water agar plates wetted with PJM medium. The *Pseudomonas* bacterium served as the amoebal food source. On 3rd day, the amoebae were washed from the surface of the agar plates and inoculated into the soils using a syringe (900 amoebae g^{-1} oven dry soil). The protozoan inoculation added negligible numbers of bacteria to the soils. PJM was added to protozoa-minus soils (controls). The soils were then gently mixed and packed to the appropriate bulk densities (Table 1). Cup assemblies were returned to the glass jars and incubated in a dark room at 22°C . Soil matric potential was -0.03 MPa (~ 9.7 μm) water-filled pore diameter) after protozoa inoculation, which corresponded to 57, 48 and 36% of water-holding capacity for soil A, Soil B and Soil C, respectively.

Sampling:

Microcosms from each soil type were destructively sampled on 0,3rd,5th ,8th ,13th ,22nd and 38th days. Mineral N and bacteria population measurements were taken from day 0 onwards. CO_2-C, CO_2-^{14}C, total soil ^{14}C, total soil ^{15}N and mineral ^{15}N measurements were taken from 3rd day onwards. Protozoa number measurements were taken from 5th day onward (major sampling dates). Bacterial populations were not measured on 13th day. No. C or N measurements were obtained for the protozoa-plug treatment from soil C on 38th day.

At sampling, the cup assemblies and NaOH concentrations were removed from the glass jars. The soil from each cup was transferred to a sterile beaker and mixed before subsamples were removed for bacterial plate counts, protozoa MPN determination and mineral N extractions. The remaining soil air-dried and ground for total soil C, total soil ^{14}C, total soil N and total soil ^{15}N analysis. Soil moisture measurements indicated that moisture loss during the experiment was negligible.

Bacterial Plate Counts:

Twenty grams of moist soil were added to a sterile 300-ml.. Erlenmeyer flask containing fifteen 2-mm. glass beads and 90 ml.. of PJM. The flask was mixed on a shaker (220 rpm) for 10 min. and then 10 ml.. of the suspension was removed to make a 10-fold dilution series (to 10^{-7}) in phosphate saline. Three replicates microcosms were used for plate counts for soil A and soil B. Two replicate microcosms were used for soil C. Two milliliter aliquots of the soil suspension from each microcosms water also transferred to two 20-ml.. test tubes for protozoa determination. Triplicate aliquots (0.1 ml..) of diluted soil suspensions were spread on each of three 0.1 strength tryptone soy agar plates. The plates were inverted and incubated for 6 days at 22°C before counting. Fungal contamination checks were made on potato dextrose agar plates containing 120 ppm streptomycine.

Protozao MPN Determination :

Protozoa were determined using the microtiter plate MPN method of Darbyshire *et al.* (1974) with modification. One of the two 2-ml. soil suspensions obtained form each microcosm soil was diluted to 6 ml. with PJM. The other 2ml. suspension (heat-treatment) was placed in a 45°C water bath for 19 min. before being diluted to 6 ml. with PJM. Heat treatment kills active protozoa but allows cyst forms to survive. The soil suspensions were vortexed for 5 S and then placed into a sterile reservoir. An 8-tip 25-µl. microdiluter was immediately dipped into the soil suspension and then placed into the first row of wells on the microtiter dish. One hundred microliters of PJM was previously added to each of the mcirotiter wells. The microdiluter was then used to dilute fivefold form the first to the eleventh row of wells. All wells were then inoculated with 25 µl. of *Pseudomonas* which served as a food source for the protozoa. Bacteria was cultured for a day before use in PJM. They were then washed by centrifugation at 7000xg and diluted 8-to 10-fold in PJM with 0.01% peptone before addition. The 12th row of wells served as a check for bacterial culture contamination or PJM contaminations. The culture wells were incubated 8 days in the dark at 15°C and then screened using an inverted microscope at 200-400 X magnification. The culture wells were then incubated for 8 days at 22°C before negative wells were rescreened. The MPN of total protozoa (no heat treatment) and of encysted protozoa (heat treatment) were calculated by using a microcomputer program developed by Clarke and Owens (1983).

Soil Respiration:

Five milliliters and 0.5-ml. aliquots of the 0.5 M NaOH were used for analysis.The 5-ml. aliquot was titrated with 0.1N HCl to determine the CO_2-C evolved from the soils. The 0.5-ml. aliquot was mixed with 12ml. of Packard HIONIC flour in a 20-ml. scintillation vial. ^{14}C was determined by counting for 20 min. in a pakard Tri-carb 2000 CA scintillation counter.

Soil C and N Analysis:

Total C was determined on ground soil by dry combustion using Leco Carbon Determinator CR-12. The quantity of ^{14}C in 100mg. soil was determined after oxidation in a Harvey. Biological Oxidizer, Model OX300. ^{14}C released during oxidation was trapped in Harvery's ^{14}C cocktail and counted for 20 min. in a Packard Tri-Carb 2000 CA scintillation counter. Recovery of ^{14}C as CO_2 plus soil C was generally greater than 90%,

Soil mineral N was extracted by mixing 20g soil with 100 ml. 2 *M* KCl soil solution for 1 h and then filtering through Whatman No. 42 filter paper. The extract was frozen before analysis. Technicon Autoanalyzers were used to measure NO_3-N (Industrial Method No. 497-77A, 1977) and NH_4-N (Industrial Method No. 98-70W, 1973).

Total soil N and all ^{15}N analysis were performed after dry combustion using a V.G. Isogas ANA-SIRA 10 system. ^{15}N analysis was performed on NO_3-N and NH_4-N obtained by microdiffusion technique (Brooks *et al.*, 1989; and Page *et al.*, 1982). NH_4-N was obtained after the addition of MgO to

convert NH_4-N to NH_3-N which diffused out to a glass fiber filter disk containing 2 *M* $KHSO_4$ to trap NH_3-N as NH_4-N. Containers were kept sealed for 7 days to allow complete diffusion of the NH_3-N out of solution. Devarda's alloy was then added to convert NO_3-N to NH_3-N. Another filter disk with 2 *M* $KHSO_4$ was placed over the solution and the containers were sealed for 7 days.

Statistical Analysis :

Analysis of variance were performed on all experimental variables using the SAS package GLM. The GLM statement LSMEANS was used to differentiate treatment means within each soil type and date (SAS ,1985).

Table(1): Selected chemical and physical properties of Ap samples form three soils.

Soil	PH[z]	Carbon %	Nitrogen %	Sand %	Silt %	Clay %	Texture	Bulk density (gcm^{-3})
A	7.7	3.20	0.63	9	46	45	Silty clay	0.86
B	7.8	2.15	0.43	37	31	32	Clay loam	0.96
C	7.5	1.01	0.32	74	9	17	Silty loam	1.03

Z 1: 2 soil: water.
Y particle size analysis by the hydrometer method.

RESULTS

Bacterial Numbers:

On day 0 bacterial numbers per gram soil were greatest in soil A and least in soil C (Fig.1). In soil B and soil C the addition of glucose, nitrogen and protozoa resulted in an increase of bacteria until 5^{th} day but this was not observed in soil A. On 5^{th} day, bacterial numbers in all three soils were approximately $4x10^8$ g^{-1} soil. After 5^{th} day the protozoa-plus treatment had significantly lower bacteria than the protozoa-minus treatment in all three soils (Fig.1, Table 2). During this time bacteria declined in all soils and in both treatments. The greatest reduction of bacteria due to protozoan grazing occurred between 5^{th} and 8^{th} days . Compared to the protozoa-minus treatment, bacteria in the protozoa-plus treatment were reduced by 68, 50, and 75% in soil A, soil B and soil C, respectively.

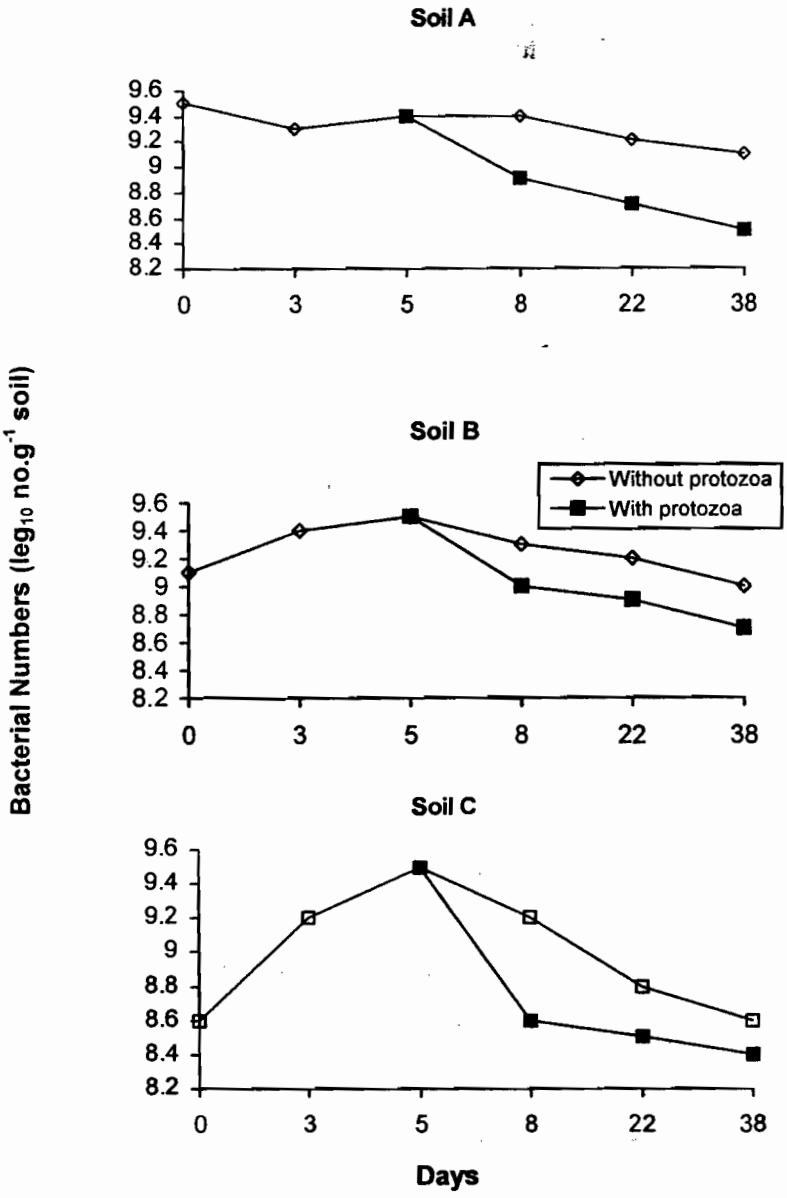

Fig. 1: Bacterial numbers in the presence and absence of protozoa in the three soils (for this and following figures the difference between treatment means within a soil and day is significant at: +, $P \leq 0.10$;*, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$; vertical bars represent ±1 standard error).

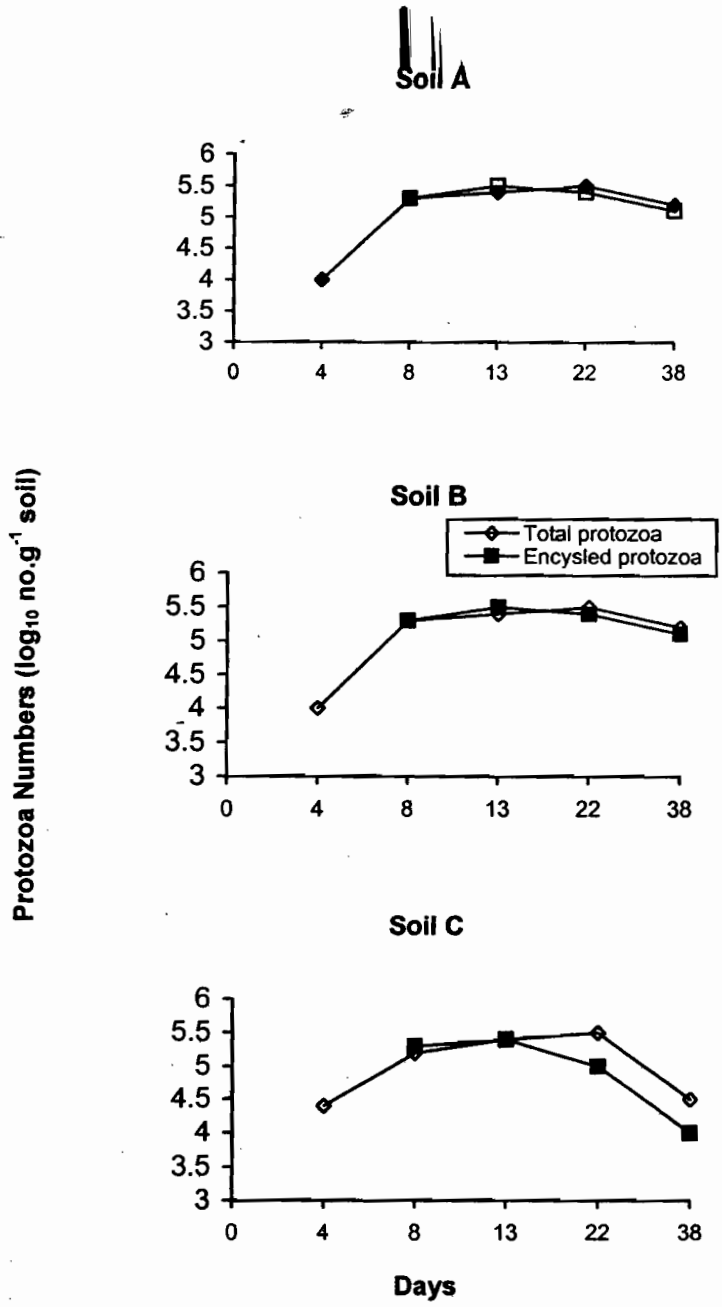

Fig. 2: Total and encysted protozoa numbers in the three soils.

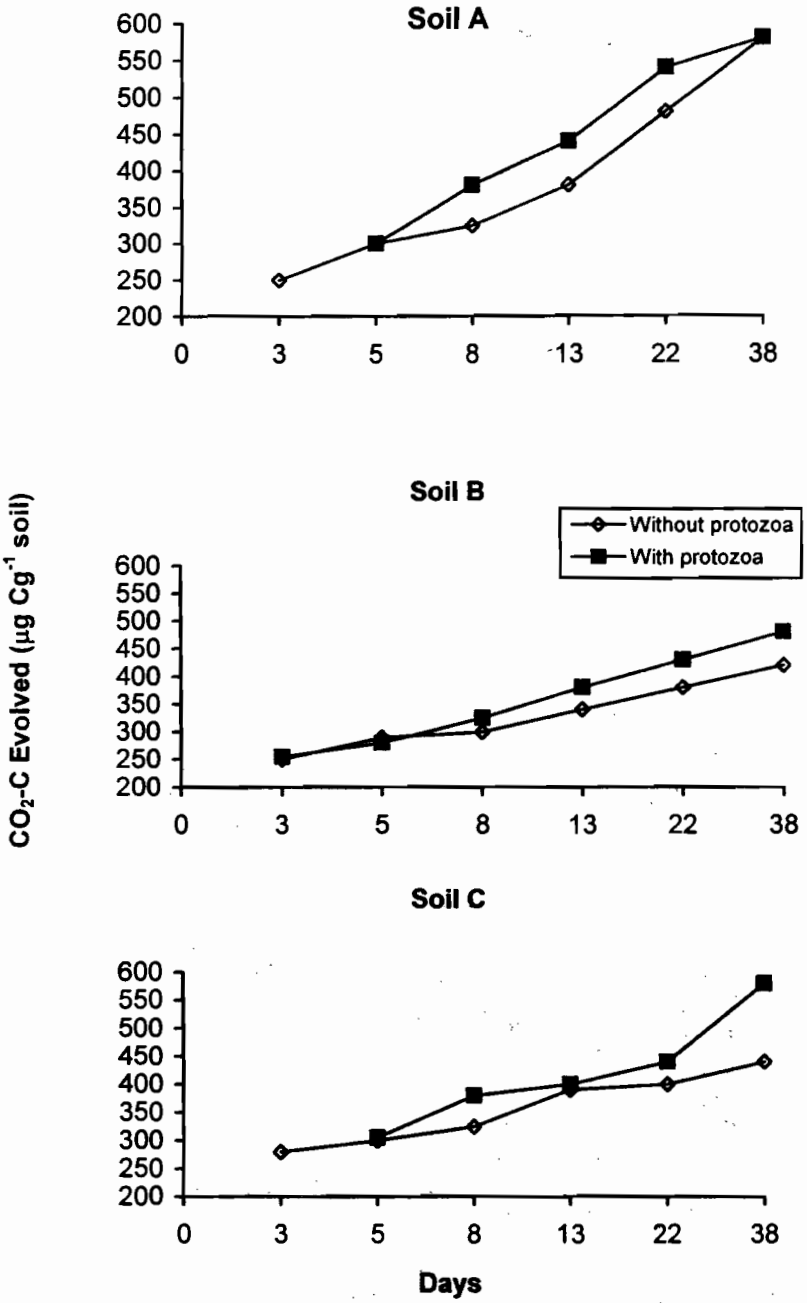

Fig. 3: CO_2 –C evolution in the presence and absence of protozoa from the three soils.

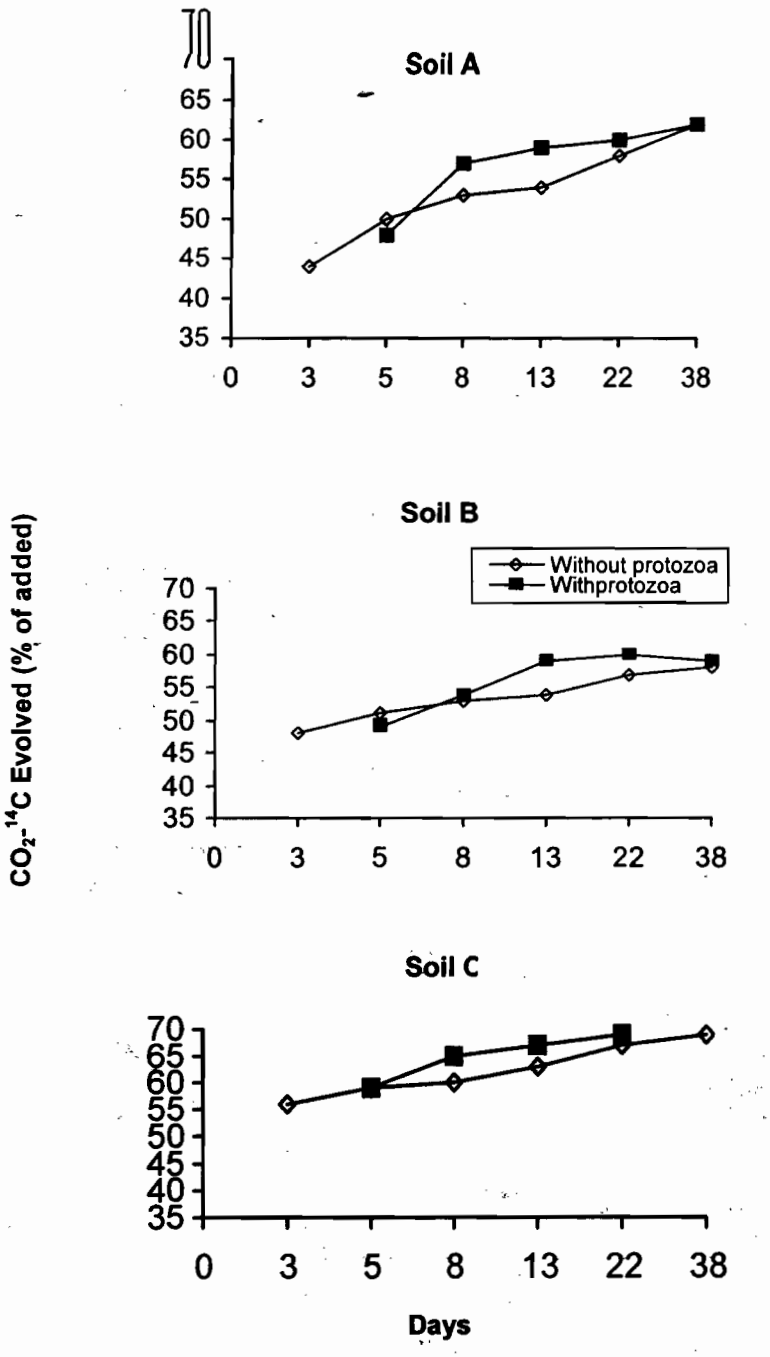

Fig. 4: CO_2-^{14}C evolution in the presence and absence of protozoa from three soils.

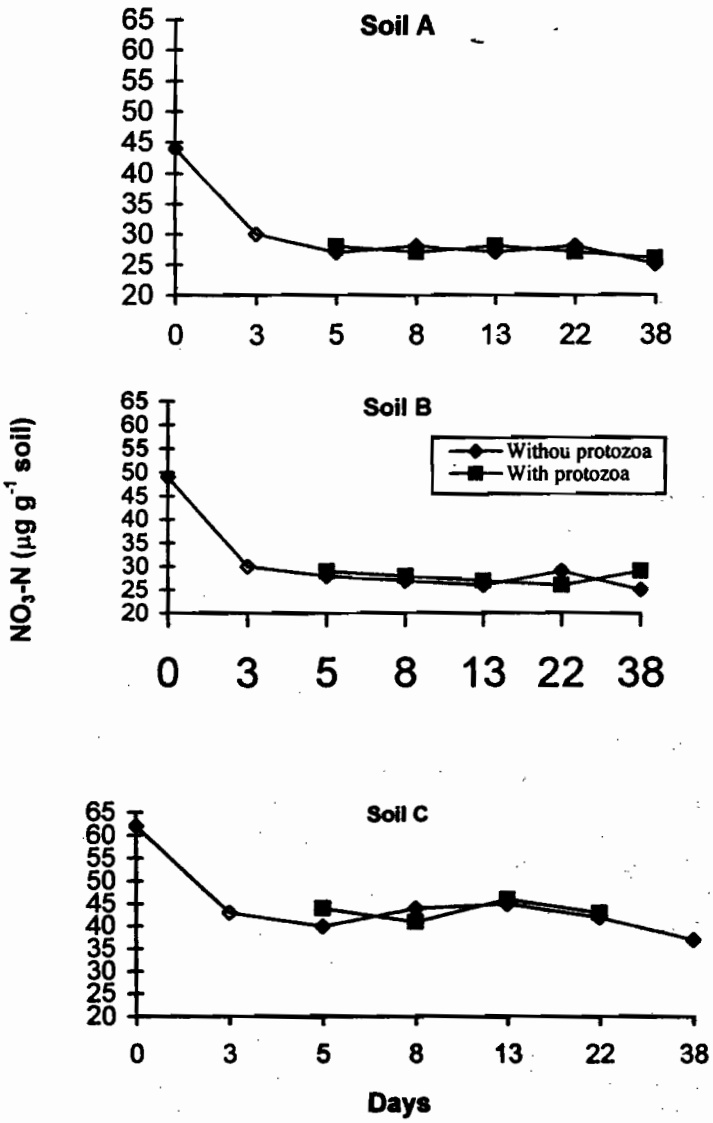

Fig. 5:NO_3-N concentration in the presence and absence of protozoa in the three soils (treatment means were not significantly different).

Table (2): Summary of ANOVA[z] for bacteria numbers (log 10 no. g^{-1} soil), protozoa numbers (log 10 no. g^{-1} soil), and CO_2-C evolution (mg C100g^{-1} soil).

Source of variation	Bacteria	Protozoa	CO_2-C	CO_2-^{14}C
Soil (s)	***	***	***	***
Treatment (T)[y]	***	*	***	***
S x T	+	NS	NS	NS
Day (D)	***	***	***	***
S x D	**	**	***	NS
T x D	***	+	***	**
S x T x D	*	NS	***	NS

Z The difference between means is significant at : +, $P \leq 0.10$; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$; NS, Not significant.

Y There were tow treatments (heat and no heat) to separate the total protozoa from cysts . In the case of bacteria, CO_2-C and CO_2-C and CO_2-^{14}C, the treatments were presence and absence of protozoa.

Protozoa Numbers:

Protozoa numbers in all three soils followed similar trends (Fig.2, Table 2). The difference between total protozoa (no-heat treatment) and encysted protozoa (heat treatment) was active protozoa. On 5, 3 days after protozoa inoculation, all measurable protozoa were active . Numbers were 10341, 4772 and 15393 g^{-1} soil for soil A, soil B and soil C, respectively. Between 5th and 8th days, the period of greatest bacterial decline, total protozoa increased greatly to 150491, 96172 and 192113 g^{-1} for three soils, respectively; however, most protozoa became encysted by 8th day. Active protozoa gradually increased between 13th and 22nd days, although the difference between total protozoa and encysted protozoa was often not significant due to the variability of the data. The sensitivity of the MPN method was not great enough to make an accurate estimate of active protozoa numbers after 5th day. Total and encysted protozoa in soil B and soil C declined greatly after 22 nd day, but protozoa in soil A declined only slightly.

CO_2 - C Evolution :

In all soils the addition of protozoa significantly increased CO_2-C evolution per gram of soil (Fig. 3 and Table 2) relative to the protozoa-minus treatment. The greatest change upon protozoa addition occurred between 5th and 8th days. On 8th day, protozoa-plus treatments were greater than protozoa-minus treatments by 16, 8 and 12% for soil A, soil B and soil C, respectively. After 8th day, CO_2-C evolution form protozoa-plus soils approximately paralleled the evolution from protozoa-minus soils. In general, CO_2-C evolution from both treatment in soil A was greater than form soil B and soil C.

CO_2 -^{14}C Evolution :

In the first 3 days, 44,47 and 56% of glucose ^{14}C added to soil A, soil B and soil C, respectively, was evolved as CO_2-C (Fig.4). Over the incubation period more CO_2-^{14}C was evolved from soil C than from the other soils.

Protozoa quickly increased CO_2-^{14}C evolution from all soils (Fig.4, Table 2). CO_2-^{14}C evolution generally increased throughout the incubation in protozoa-minus treatments. In contrast, Co_2-^{14}C evolution from protozoa-plus treatments increased very slightly after 8th day for soil C and after 13th day fsor soil A and soil B (Fig. 4). The amount of CO_2-^{14}C evolved was similar for both treatments at the end of incubation (57-67%) of added ^{14}C).

Between 44 and 56% of ^{14}C-glucose was transformed into bacterial C and bacterial metabolites after 3 days.

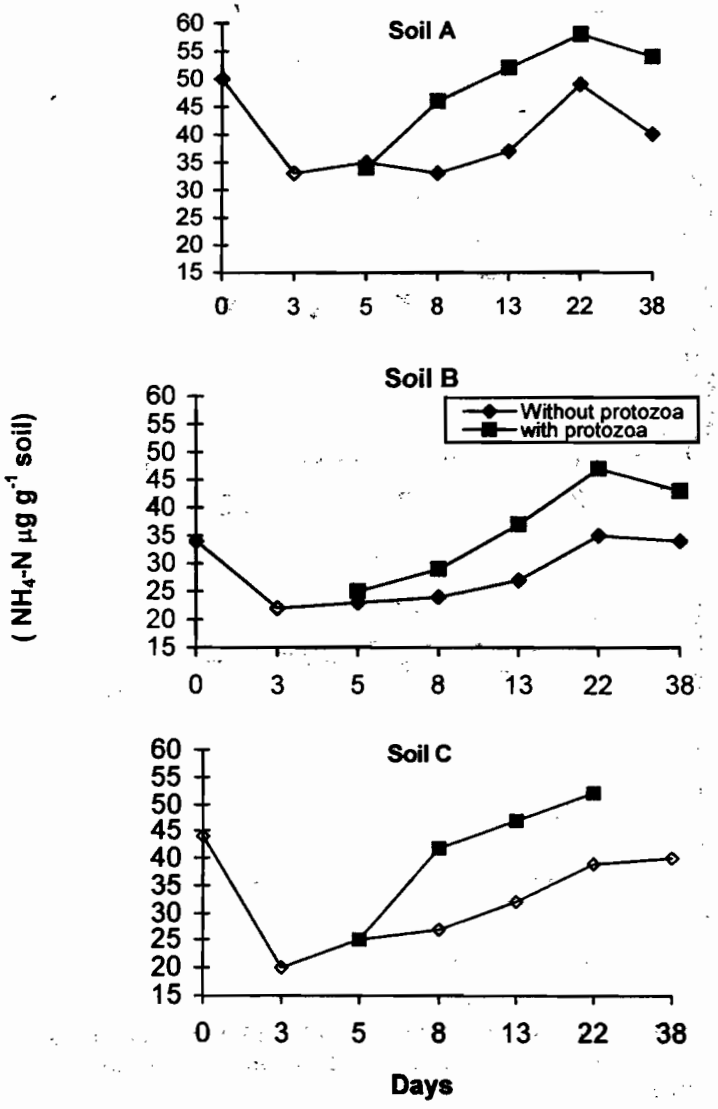

Fig.(6): NH_4-N concentration in the presence and absence of protozoa in the three soils

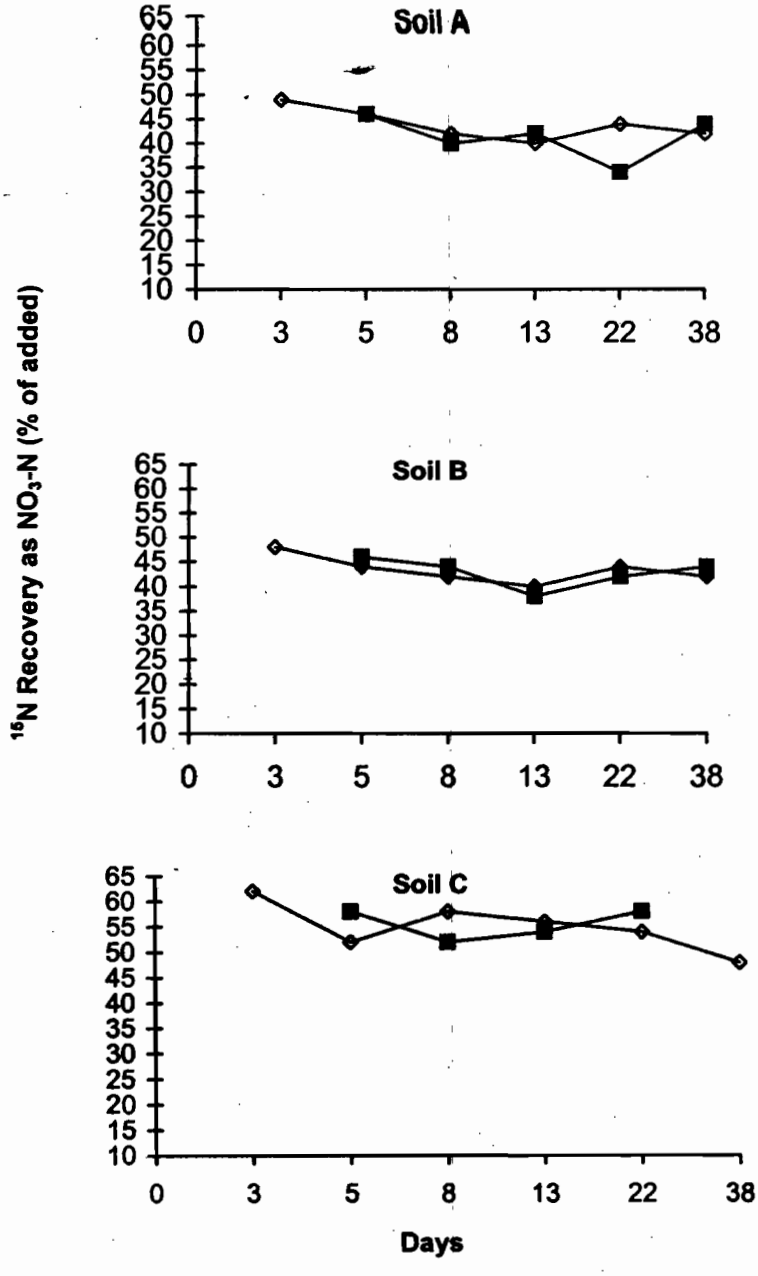

Fig. (7): No_3-^{15}N recovery in the presence and absence of protozoa in the three soils (treatment means were not significantly different).

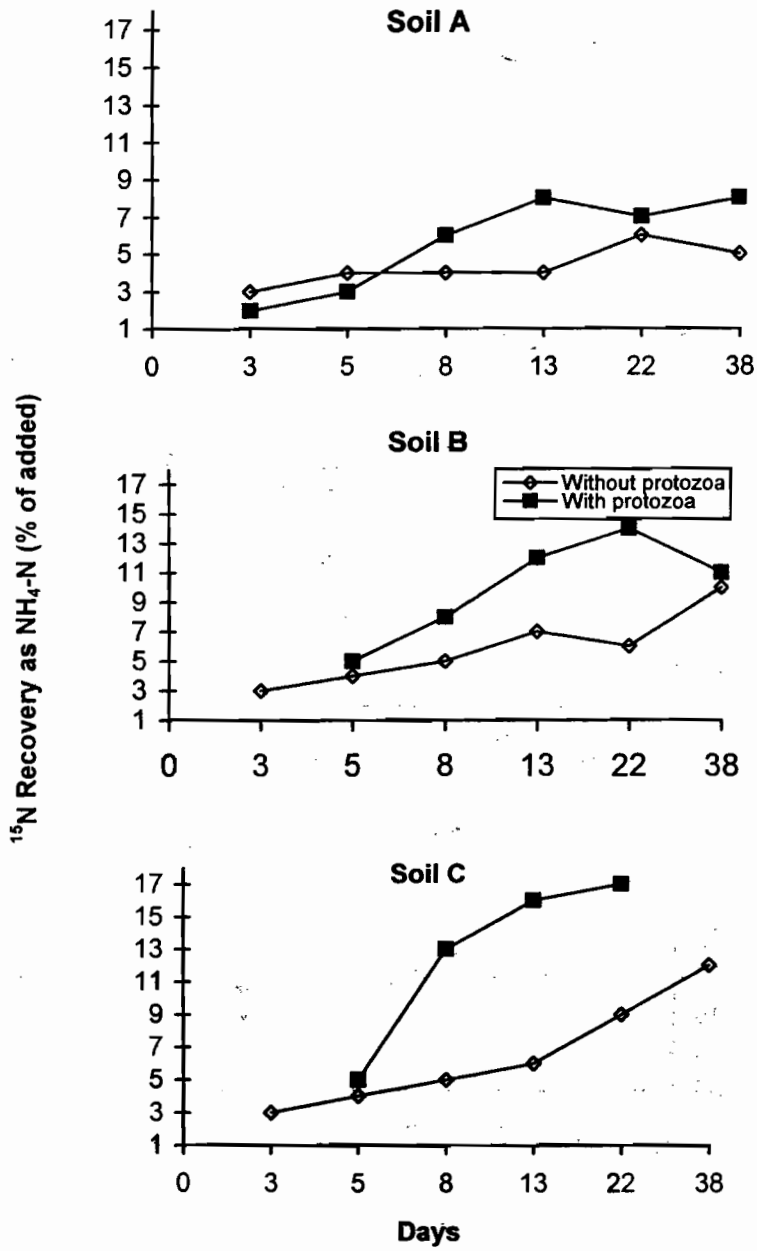

Fig.(8): NH_4-^{15}N recovery in the presence and absence of protozoa in the three soils

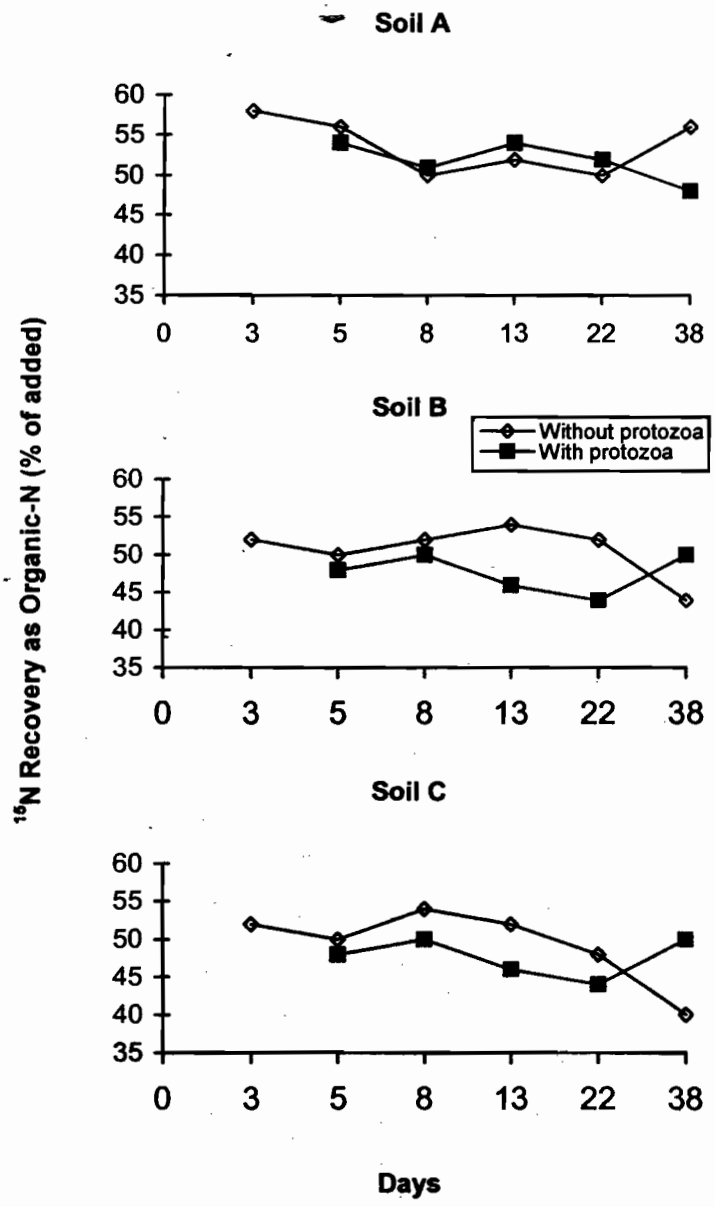

Fig. (9): Total ^{15}N recovery in the presence and absence of protozoa in the three soils

Table (3):Summary of ANOVA[z] for NO_3-N and NH_4-N (μgNg^{-1} soil), and NO_3-N^{15}, NH_4-^{15}N, and Organic-^{15}N Recovery (% of added ^{15}N).

Source of variation	NO_3-N	NH_4-N	NO_3-^{15}N	NH_4-^{15}N	Organic-^{15}N
Soil (s)	***	***	***	***	***
Treatment (T)[y]	NS	***	NS	***	**
S x T	NS	*	NS	***	NS
Day (D)	***	***	***	***	*
S x D	+	+	+	***	NS
T x D	NS	***	NS	***	NS
S x T x D	NS	NS	NS	***	NS

Z The difference between means is significant at : +, $P \leq 0.10$; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$; NS, Not significant.

Y treatments were presence and absence of protozoa.

Protozoa inoculation decreased bacterial numbers rapidly between 5th and 8th days, thus the effect of water and C amendment, and inoculation lasted up to 13th day. Less than 3% additional ^{14}C was evolved in the protozoa-plus treatments between 13th and 38th days. This represents organism activity in moist unperturbed soils. The ^{14}C remaining in soil on 38th day was assumed to be incorporated into bacteria, amoebae and newly formed organic matter.

Mineral N Concentration :

On day 0, NO_3-N (Fig. 5) was greatest in soil C (62 $\mu g\ g^{-1}$ soil) and least in soil A (45 $\mu g\ g^{-1}$ soil) after KNO_3 addition (31 $\mu g\ g^{-1}$ soil). Glucose amendment resulted in net NO_3-N immobilization of 14, 19 and 20 μgNg^{-1} soil, for soil A, soil B and soil C, respectively, by 3rd . NO_3-N in soil A and soil B decreased slightly until 8th day and then became relatively stable. After 22nd day NO_3-N decreased slightly in all soils. NO_3-N was not significantly different between treatments (Table 3).

On day 0, NH_4-N concentration (Fig. 6) was greatest in soil A (51 $\mu g\ g^{-1}$ soil) and least in soil B (35 $\mu g\ g^{-1}$ soil). Glucose amendment resulted in net NH_4-N immobilization of 18, 13 and 24 $\mu g\ Ng^{-1}$ soil for soil A, soil B and soil C, respectively, by 3rd day. Net mineralization of NH_4-N occurred from 3rd to 22nd days in all soils and treatments, although NH_4-N in the protozoa-minus treatment in soil A increased slightly until 22nd day. After 22nd day NH_4-N concentration was stable or decreased slightly.

Protozoa addition significantly increased net NH_4-N mineralization in all three soils (Table 3). The greatest increase occurred between 5th and 8th days. On 8th day, protozoa-plus treatment concentrations exceeded protozoa-minus concentrations by 33, 29 and 58% for soil A, soil B and soil C, respectively.

Partitioning of KNO_3-^{15}N:

On day 3,3 days after amendment, ^{15}N recovered as NO_3-^{15}N (Fig. 7) was 49, 46 and 62% for soil A, soil B and soil C, respectively. Between 5th

and 8th days NO_3-^{15}N decreased slightly in soil A and soil B, and in the protozoa-plus treatment of soil C. After 8th day, recoveries varied slightly with time, except for the protozoa-minus treatment of soil C which gradually decrease until 38th day.

On 3rd day, ^{15}N recovered as NH_4-^{15}N (Fig. 8) was 2.1, 3.4 and 2.9% for soil A, soil B and soil C, respectively. Recovery of NH_4-^{15}N generally increased with time, although the protozoa-minus treatment in soil A did not increase until after 13th day. NH_4-^{15}N either did not change, or it decreased slightly after 22nd day in soil A and soil B. NH_4-^{15}N in soil C increased throughout the measurement period.

In all soils the addition of protozoa significantly increased net NH_4-^{15}N mineralization relative to the protozoa-minus treatment (Table 3). The greatest rate of net NH_4-^{15}N mineralization occurred between 5th and 8th days. On 8th day, NH_4-^{15}N in the protozoa-plus treatment exceeded the protozoa-minus treatment by 110, 80 and 200% in soil A, soil B and soil C, respectively

The non-mineral ^{15}N remaining in the soils was assumed to be in the microorganisms, or their by-products, hence this pool was called organic ^{15}N. Organic-^{15}N (Fig. 9 and Table 3) was greatest in soil A and least in soil C. IN soil B and soil C the protozoa-plus treatment had significantly lower recoveries of organic ^{15}N than the protozoa-minus treatment on some sampling dates.

DISCUSSION

The results of this study will be discussed for two time periods: (i) a period of rapid changes between 0 and 13th days due to the inoculation of organisms and amendment of C and N to soils, and (ii) a period of slower changes from 13th to 38th days.

Effect of texture on dynamics of carbon and nitrogen during the first 13 days after addition of substrates :

All soils showed reduced bacterial numbers due to protozoan grazing. The coarse-textured soil showed the largest decrease of bacterial and concurrent increase in protozoan numbers between 5th and 8th days. After 8th day, bacterial numbers in the protozoa-plus treatment declined slightly in soil C but more rapidly in soils A and B. Protozoa in soil C may have preyed upon relatively more bacteria and at a higher rate than the protozoa in the fine-textured soils. Bacterial consumption by protozoa can be calculated by dividing the difference between bacterial numbers in the grazed and ungrazed microcosms by the number of protozoa in the soil. The method is approximate because bacterial growth rates are assumed to be the same in both treatments and bacteria are assumed not to regrow and replace the consumed soils (Gupta and Germida ,1989; EL- sayed, 2002, a, b & C; El-Sayed and Abo – EL- Wafa, ,2001). Between 3rd and 8th days, the consumption of bacteria by protozoa in soil A, soil B and soil C was 11421, 7312 and 15213 bacteria per protozoa, respectively .

CO_2-C evolution:

in all protozoa-plus soils increased greatly over the control between 5th and 8th days reflecting increased grazer activity thus supporting the findings of other researchers (Gupta and Germida, 989; and Kuikman *et al.* ,990; El- Sayed and Abdel - Mawly, 1999 a & b; and Abdel- Mawly and El – Sayed, 1999). The rate of CO_2-C evolution was very similar between treatments after 8th day, indicating that protozoa had a marginal effect at increasing C mineralizing after this time.

The CO_2-^{14}C results showed differences that confirmed a texture effect for C originating from the bacterial biomass. The amount lost as CO_2-^{14}C was greatest from soil C and least from soil A. All soils showed increases of CO_2-^{14}C evolution due to grazing but the evolution from soil C reached a maximum by 8th day compared to 13th day for soil A and soil B. This paralleled the great decrease in bacterial numbers induced protozoan grazing between 5th and 8th days in soil C. A portion of the immobilized ^{14}C was non-mineralizable. The protozoa contributed to labile soil ^{14}C which was rapidly reutilized by soil bacteria.

The N results provided further support for the hypothesis that the frequency of protozoan grazing was greater in the coarse-textured soil than in the fine-textured soils. NH_4-N and N_3-N concentrations decreased as N was immobilized due to the glucose amendment. A total of 31, 31 and 43 μg N g^{-1} soil was immobilized between 0 and 3rd days for soil A, soil B and soil C, respectively. The bacteria in soil C probably immobilized more N because amendment addition caused a greater increase in bacterial numbers than in the other two soils. Mineralization of N as NH_4-N in the protozoa-minus treatment occurred more rapidly in soil C, indicating that bacterial turnover in this soil had resulted in a larger surplus of N than in the other soils. In the absence of fauna, N mineralization is determined by the consumption rate of the substrate, the C:N ratio of the substrate and the bacteria, and the C utilization efficiency of the bacteria (Robinson *et al.* 1989; and El- Sayed, 1997 a & b; Faragallah and El-Sayed, 2002).

When protozoa were present, NH_4-N mineralization increased relative to the protozoa-minus treatment in all soils. Increased N mineralized and bacterial N turnover due to the presence of protozoa, with or without the presence of plants, has been reported by many authors (Ritz and Griff iths , 1987; Couteaux *et al.*, 1988; Kuikman and Van Veen ,1989; and Kuikman *et al.*, 1989 and 1991 and ; El-Sayed, 1998 a, b,c &d). Soil C had the greatest percent increase in net NH_4-N mineralization relative to the protozoa-minus treatment. Thus protozoa were more effective at mineralizing the recently immobilized N in the coarse-textured soil.

Less NO_3-^{15}N was immobilized between 0 and 3rd days in soil C than in soil A and soil B because soil C had more non-labelled NH_4-N plus NO_3-N on day 0, the day of amendment addition. Hence the label was subject to more dilution in soil C. NO_3-^{15}N remaining in soil did not differ between treatments within each soil and date, indicating that addition of protozoa did not affect the immobilization of NO_3-^{15}N into the bacterial biomass. NH_4-^{15}N mineralization in the protozoa-minus treatment showed the greatest increase

with time in soil C, compared to the other soils, which complements the greater evolution of CO_2-^{14}C in this soil. This support the findings of Dinwoodie and Juma (1988), Rutherford and Juma (1989a,b) and El-Sayed (1999 a & b) the turnover of C and N within the microbial biomass is faster in coarse-textured soils than in fine-textured soils.

Even though some organic ^{15}N would have been present as active ^{15}N at the time of protozoa addition, most ^{15}N on 3rd day would have been present as bacterial ^{15}N (Voroney and Paul ,1984 and ; El-Sayed and Hegab, 2001 a & b). The increased net NH_4-^{15}N mineralization in the presence of protozoa may have originated mainly from bacterial ^{15}N since the experiment was not conducted under conditions of mineral N limitation. Under conditions of N limitation protozoan grazing would increase bacterial metabolic activity (Robinson *et al.*, 1989 ; and El- Sayed, 1995 a , b & C). thereby increasing the decomposition of soil organic matter of plant residues, resulting in the increased meneralization of ^{15}N from the active N phase. The latter concept was not examined in this study.

Protozoa had the greatest effect of increasing net NH_4-^{15}N mineralization in soil C (Fig. 7); therefore, protozoa were more effective at mineralizing bacterial N in this soil than in the fine-textured soils. On 8th day the percent increase in NH_4-^{15}N mineralizaiton, compared to the protozoa-minus treatment, was much greater in soil C (200%) than in soil A (100%) and soil B (80%). The time required to reach the maximum concentration of NH_4-^{15}N was greater than the time needed for maximum CO_2-^{14}C evolution because of the internal cycling which occurs for soil ^{15}N. In contrast, soil ^{14}C is not recycled once it is mineralized to CO_2-^{14}C.

The effective grazing of bacteria by protozoa in soil C can also be seen if one considers the proportion of organic-^{15}N which is mineralized to NH_4-^{15}N. If the increase in NH_4-^{15}N between 5th and 8th days is divided by the organic-^{15}N present on 5th day, less than 1% of organic-^{15}N is mineralized in the three protozoa-minus soils; however, 6,7 and 23% is mineralized in the protozoa-plus treatment for soil A, soil B and soil C, respectively. Hence, the protozoan grazing of bacterial N was most effective in soil C.

Effect of texture on dynamics of carbon and nitrogen 13-38 days after amendment addition :

The effect of texture on protozoan grazing was not very marked after 13th day. During this period the influence of glucose amendment had ceased and changes in bacterial and protozoan populations, and C and N dynamics were reduced. Protozoa had little effect in further reducing bacterial numbers and the majority of amoeba encysted. CO_2-C evolution continued to increase in all soils but the rate of evolution for each soil was unaffected by the presence of protozoa. CO_2-^{14}C continued to increase in the protozoa-minus treatment for each soil but evolution from the protozoa-plus treatment was very slight. CO_2-^{14}C evolved for both treatments was similar late in the incubation suggesting that a maximum amount of soil ^{14}C is lost as CO_2-^{14}C from each soil, and that protozoa affected the rate, but not the quantity of soil ^{14}C that was lost. NH_4-N and NH_4-^{15}N concentrations in both treatments increased slightly or decreased due to net N immobilization .

The influence of protozoa was reduced during the latter part of the experiment because glucose induced bacterial growth had ceased and protozoa had reduced bacterial numbers greatly during the first 13 days. The ^{14}C and organic-^{15}N remaining in soil was contained within a protected bacterial population, active or inactive protozoa or microbial by-products. Active protozoan grazers could not access much of this ^{14}C or ^{15}N. NH_4-^{15}N continued to increase in both treatments for a longer period of time than CO_2-^{14}C evolution because N cycles internally within the soil. The death of some protozoa and bacteria after 22nd day supplied a readily available C source for the remaining bacterial population resulting in some immobilization of mineral N and ^{15}N.

CONCLUSION

The results of this study support the hypothesis that protozoa increase the mineraliztion of bacterial C and N, and this effect is most apparent in coarse-textured soils. This effect may partially explain the greater turnover of C and N in coarse-textured soils compared to fine-textured soils. This study also showed that protozoan-induced effects are transient in soil. Kuikman *et al.* (1990) also found that protozoa only stimulated C turnover during the initial stages of a laboratory incubation. In nature, the greatest effects of protozoa on bacterial turnover and mineralization probably occur briefly after perturbation, such as aggregates disruption, energy addition (Ritz and Griffiths, 1987) or water addition (Hunt *et al.*, 1989; and Kuikman *et al.*, 1991). Thus, protozoa probably are ecological important in nature during the brief periods following perturbations which stimulate microbial growth. Soil protozoa also have rapid encystment-excystment abilities, as was observed in this study, to help survive changes in the soil environment (Bamforth, 1988; and El-Sayed and Salem, 2002).

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العلاقة ما بين قوام التربة ومعدل معدنه النيتروجين والكربون والكائنات الحية الدقيقة بالتربة

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أثر قوام التربة على المسافات البينة وتعداد الحيوانات الأولية والبكتيريا وأنشطتها فى التربة. الهدف من هذه الدراسة اختبار نظرية أن الحيوانات الأولية التى تلتهم وتتغذى على البكتيريا تزيد من معدنة الكربون والنيتروجين البكتيرى فى التربة ذات القوام الخشن أكثر من التربة ذات القوام الناعم. هذه التجربة لقوام التربة وكائناتها الحية الدقيقة تتكون عيناتها من ثلاث أنواع من التربة المعقمة الطينية السلتية (SlC) والتربة الطينية اللومية (CL) والتربة السلتية اللومية (SL) الملقحة ببكتيريا البزيدوموناس *Pseudomonas* والمعاملة بمعاملتين إحداهما ملحقة بالحيوانات الأولية والأخرى بدونها ومواعيد أخذ العينات خمسة تواريخ من المعاملة. تعداد بكتيريا البزيدوموناس إضيف له فى مكان تجربتها الجلوكوز ونترات البوتاسيوم فى يوم التجربة (0 day). أما الحيوان الأولى أنثاميبا *Acanthamoeba* أضيف إلى أنواع التربة الثلاث. فى اليوم الثالث من المعاملة البكتيرية. أعداد البكتيريا فى اليوم الخامس فى أنواع التربة الثلاث كانت حوالى ٤ × ١٠^{٨} جرام$^{-١}$ فى التربة. الانخفاض الكبير فى البكتيريا كان نتيجة تغذية الحيوان الأولى ما بين اليوم الخامس والثامن. أنواع التربة الثلاث أظهرت زيادة فى انتشار الكربون (CO_2-^{14}C) ومعدنة النتروجين (NH_4-^{15}N) نتيجة تغذية الحيوان الأولى ولكن معدل المعدنة المسجل للنتروجين فى التربة السلتية اللومية SL كان أكبر بكثير عن المسجل فى الأراضى ناعمة القوام الطينية اللومية ، الطينية السلتية (CL,SlC). تأثير القوام على تغذية الحيوان الأول لم تلاحظ وتشاهد فيما بين اليوم الثالث عشر (١٣) والثامن والثلاثون (٣٨) حيث إنه مبكر فى الحضانة وأن تأثيرات الحيوان الأولى المحققة كانت عابرة ومؤقتة فى جميع أنواع قوام التربة التي درست وكانت أكثر ظهورا فى الأراضي ذات القوم الخشن.