SOIL MICROBIAL POPULATIONS AND ACTIVITIES IN REDUCED CHEMICAL INPUT AGROECOSYSTEMS[1]

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ABSTRACT

Crop management systems which reduce chemical inputs represent attempts by researchers and farmers to find a way to sustain productivity while reducing costs, protecting human health and maintaining the resource base. This study, conducted in the Piedmont of North Carolina, was initiated to determine how reductions in nitrogen (N) fertilization, and the use of crimson clover (*Trifolium incarnatum* L. var Tibbee) as a green manure would affect populations and activities of soil microorganisms. Four continuous corn (*Zea mays* L.) treatments were used: no-till (receiving herbicides and soil insecticides) with 0 to 140 kg N ha^{-1} as NH_4NO_3; conventionally tilled receiving 140 kg N ha^{-1} but no pesticides; and conventionally tilled with a crimson clover winter green manure but no fertilizer or pesticides. Populations were determined using selective media for total bacteria, Gram-negative bacteria, fungi, actinomycetes, *Bacillus* spp., and *Pseudomonas* spp.. Microbial activities were estimated by enzyme assays for acid and alkaline phosphatase, arylsulfatase, and β-glucosidase. Microbial biomass carbon (C) was determined following chloroform fumigation-extraction procedures and levels of available N were measured by an anaerobic incubation procedure. Surface

soil (0 - 7.5 cm) from the no-till treatment receiving 140 Kg N/ha contained significantly more fungi than did soil from the non-fertilized, no-till treatment. Microbial biomass C and available N were not affected by N addition, but activities of acid phosphatase and β-glucosidase were significantly higher in the fertilized soil than in the non-fertilized soil. Surface soil from a crimson clover/corn rotation contained significantly larger populations of *Bacillus* spp. (260% more), actinomycetes (310% more), and total bacteria (120% more) than did soil from the well-fertilized conventionally tilled, no pesticide treatment. In addition, microbial biomass C, available N, and activities of alkaline phosphatase, arylsulfatase, and β-glucosidase were significantly higher in surface soil in the crimson clover treatment compared to no clover surface soil. Although the soil biological properties changed significantly during the year, values remained constant relative to each other. Microbial numbers and activities were highest in the spring (peaking approximately in May), lowest in September, and reached a secondary peak in the fall (approximately in November). The effect of N fertilizer on soil biological properties deeper in the profile (7.5 - 30 cm) was similar to the effect in the soil surface. However, the numbers and activities of the soil microflora at a depth of 7.5 - 30 cm generally did not significantly differ between the crimson clover treatment

and the conventionally tilled, N fertilized treatment. Incorporation of a crimson clover green manure as an alternative to inorganic N fertilization appears to optimize the internal cycling efficiency of the cropping system and maximize use-efficiency of external resources.

INTRODUCTION

During the last 50 years, farmers in the United States have dramatically increased crop yields through the use of fertilizers, pesticides, and improved varieties (National Research Council, 1989). Today, the rising costs of chemical inputs and a host of environmental concerns have caused farmers to consider alternative agricultural methods to reduce costs and to protect human health and the resource base. Some of these methodologies include using crop rotations, leguminous winter cover crops as green manures, reducing tillage, adding animal manures to the soil, and integrated pest management procedures.

Changing the crop management system will change the soil microclimate and affect the soil biota (Paul and Clark, 1989). Changes in the soil microflora may have significant implications regarding the productivity and sustainability of reduced chemical input (RCI) agroecosystems. Such alternative systems represent an attempt to optimize the internal cycling efficiency of nutrients and to maximize the use-efficiency of external resources (Buchanan, 1990). Although microorganisms make up only 1 - 8 % of the soil organic matter they influence crop production by acting as catalysts for biotransformations (Roder et al., 1988). Through the processes of decomposition, immobilization, and mineralization, soil microorganisms control the flow of carbon (C), nitrogen (N), phosphorus (P), and sulfur (S) through the terrestrial ecosystem (Sarathchandra et al.,

1988). As farmers consider conversion to RCI systems they must consider the effect of these systems on soil microflora.

A common RCI system is the incorporation of a leguminous green manure into the soil to supply N and organic matter. This practice serves as an alternative to inorganic N fertilization. Bolton et al. (1985) showed that the incorporation of a leguminous green manure increased soil microbial biomass and enzyme activities as compared with soil which had been inorganically fertilized. Soil from a rotation of oats (*Avena sativa* L.) and clover (*Trifolium pratense* L.) was found to have significantly greater microbial biomass, bacterial and fungal populations, dehydrogenase activity, and CO_2 evolution as compared with soil from a system of continuous corn (Fraser et al., 1988).

Fertilization affects the soil microbial biomass by increasing root biomass, root exudates, and crop residues and thus provides increased substrate for microbial growth. Martyniuk and Wagner (1978) found microbial populations were greater in fertilized soil than in non-fertilized soil. Sarathchandra et al. (1988) found that N fertilization increased levels of organic C in the soil. Any management practice that increases total C accumulation should also increase the size and activities of the soil microbial biomass (Buchanan, 1990).

Roder et al. (1988) comment that interactions between

rotation treatments and organic soil amendments or fertilizer treatments on microbial biomass have received little attention. Therefore the study reported in this paper had as its objectives a comparison of the soil biological properties in RCI and conventional agroecosystems and a description of the seasonal variation in the soil biota. The treatments considered showed how the practices of N fertilization and including crimson clover (*Trifolium incarnatum* L. var Tibbee) in the rotation as a green manure crop would influence populations and activities of the soil microflora.

MATERIALS AND METHODS

The 6-hectare experimental site was established in the fall of 1985 in the Piedmont of North Carolina near Raleigh, at the North Carolina State University Research Unit 9. The soils at Unit 9 are Cecil and Appling gravelly sandy loams (Typic Kanhapludults) with 2 - 6% slope and moderate erosion (Table 1) and are typical of the southern Piedmont of the United States. Treatments were arranged in a randomized complete block design with four replications of each treatment based on landscape position and soil texture and were designed for a long-term study to assess the agronomic and economic feasibility of cropping systems utilizing reduced rates of chemical inputs. Four treatments were chosen for the present study (Table 2). A no-till corn monoculture system (winter fallow), which received 0% or 100% of the recommended N fertilizer (140 kg N ha^{-1} as NH_4NO_3), showed the effect of reducing N fertilization on soil biological properties. Only N fertilizer was used because soil levels of P and K were considered adequate, based on soil tests conducted by the North Carolina Department of Agriculture Agronomic Division, Soil Testing Laboratory. Reduced chemical input systems received N either in the form of inorganic fertilizer (140 kg N ha^{-1} as NH_4NO_3) or as crimson clover grown as a green manure crop. Comparing the RCI systems showed the effect of the green manure vs N fertilizer on soil biological properties. Since the no-till inorganic N treatments differed from the green

manure treatment in tillage and pesticide usage, the effect of N fertilizer vs green manure could not be compared directly.

Clover was established in early October by mowing corn residue and weeds, disking to remove ridges, and planting clover with a no-till drill at a rate of 22 kg seed ha^{-1}. Clover seed was inoculated with a commercial preparation of *Rhizobium trifolium* at planting. Clover plants were incorporated into the soil on April 9, 1990 by chisel plowing and disking. The crimson clover aboveground biomass was 4430 kg ha^{-1} containing 117 kg ha^{-1} of N and having a C/N ratio of 16.4.

Soil samples were collected from the interrow in the center of each plot every month from January, 1990 to January, 1991. Approximately 20 cores were taken by sampling every 1.5 meters with a soil probe (2-cm diameter) to a depth of 7.5 cm. During the months of May, August, and December additional samples were collected from a depth of 7.5 - 30 cm. For each sampling and plot, cores were pooled, placed in plastic bags, and taken to the lab where the soil was passed through a 2 mm sieve and stored in the dark at 4° C. In most cases, analysis began within 48 hours of sampling and all tests were completed within 2 weeks.

Soil moisture content was determined for all samples. Soil pH value was measured in a soil-water suspension (1:1, w/v). Selective media were used to enumerate populations of

soil microorganisms. Fungi were estimated using DPY medium (Papavizas and Davey, 1959) and Martin's Rose Bengal agar (1950). Fluorescent pseudomonads were estimated with the medium developed by Sands and Rovira (1970) and Simon et al. (1973). Actinomycete Isolation Agar (DIFCO) was used to enumerate actinomycetes. Total numbers of bacteria were measured by plating aliquots of soil suspension on tryptic soy agar (TSA) (Martin, 1975) while Gram-negative organisms were enumerated on TSA plates which included crystal violet (0.004%). Pasteurization of dilution tubes (10 minutes at 85° C) followed by plating aliquots on nutrient agar was used to enumerate *Bacillus* spp. (Wollum, 1982).

The activities of the soil enzymes acid and alkaline phosphatase, arylsulfatase, and β-glucosidase were measured with the method described by Tabatabai (1982).

Microbial biomass was determined using a fumigation-extraction procedure which was a modification (Vance et al., 1987; Sparling and West, 1988) of the fumigation-incubation technique developed by Jenkinson and Powlson (1976). Duplicate 15 g samples were used. After labile organic C was extracted from both the fumigated and the non-fumigated samples with 0.5 M K_2SO_4, a 4 ml aliquot of the extract was digested for 30 minutes at 150° C in the presence of 1 ml of 0.066 M $K_2Cr_2O_2$ and 5 mls of concentrated H_2SO_4. After digestion, the samples were titrated with 0.034 N $FeNH_4SO_4$ and biomass calculated from organic C values.

From September 1990 to January, 1991 soil samples were analyzed for N availability using a seven day anaerobic incubation and measuring the increase of ammonium in the sample (Keeney, 1982).

In October 1990, samples were collected to determine whether the location from which the samples were collected would affect the microbial properties measured. Samples were collected from the interrow position from which the monthly samples were taken, in the row and from a position midway between the row and the interrow. These samples were treated and analyzed in the same way as the monthly samples.

During July 1991, a study was conducted to determine whether storing soil samples for several weeks at 4° C influenced the soil biological properties measured. Samples were collected from block 2 of each treatment and handled in the same manner as the monthly samples. Starting within 24 hours of collection all tests were conducted on these samples. Measurements were made on day 1, day 7, day 14, and day 21 following sampling. Between testing times, samples were stored in the dark at 4° C.

Statistical analyses were conducted using SAS statistical packages (SAS, 1982). Analysis of variance was performed on all data using PROC GLM to perform the ANOVA procedures. Correlation coefficients were determined using PROC CORR.

RESULTS AND DISCUSSION

EFFECT OF SAMPLING POSITION ON SOIL MICROBIAL PROPERTIES

Sampling position had little effect on the measurement of microbial properties. Generally the microbial properties were greater in the row than in the interrow or the mid-way position between the interrow and row (Table 3); however in most cases the differences were not significant. While the fungal populations measured with Martin's rose bengal medium decreased significantly from the row to the interrow position, the fungal populations measured by the DPY medium did not differ significantly by position. Likewise for the soil enzymes which were greater in the row, only the arylsulfatase values were significantly greater in the row than in the other sampling positions. Microbial biomass and available N were higher in the row samples but among sampling positions the differences were not significant.

EFFECT OF STORAGE ON SOIL MICROBIAL PROPERTIES

Generally, length of storage up to 21 days at 4° C had little affect on the soil microbial properties (data not shown). Available N, microbial biomass, alkaline phosphatase, arylsulfatase, β-glucosidase, fungi, and *Bacillus* spp. remained constant during the storage period. However, total bacteria, *Pseudomonas* spp. and acid phosphatase increased during 21 days of storage. For total bacteria, as measured on tryptic soy agar (TSA), the prestored value was 7.20 as compared to the highly significantly different value

of 7.67 log cfu's / g soil for the 21 day stored sample. Although total bacteria increased significantly, the increase of the Gram negative bacteria was not significant. Gram negative bacteria ranged from 82 to 85% of the total bacteria suggesting that there was no differential effect of storage on Gram negative bacteria as compared to the Gram positive bacteria. After 3 weeks of storage *Pseudomonas* spp. had increased from 3.78 to 4.81 log cfu's / g soil and the difference was significant at the $p = 0.003$ level. Acid phosphatase increased 0.79 to 4.04 μmoles p-nitrophenol g^{-1} $min^{-1} \times 10^{-2}$ during storage. Since most assays were run within 7 to 10 days of collection, the values reported probably represent what would have been obtained had all assays been run immediately after collection and would be close to the true value. It should be pointed out that in the main part of the study on microbial properties, comparisons of assay values were always made between samples that had been assayed after the same length of time of storage. Thus while the reported values may not represent the true value, comparisons among different samples are valid for purposes of determining whether management schemes had a different effect on the soil microbial properties.

THE EFFECT OF N FERTILIZATION ON SOIL MICROBIAL PROPERTIES

Generally, N fertilization increased microbial populations. Differences between the treatments were not large and high variability among replicates often prevented

the analyses of variance from detecting significant differences. When averaged across the 13 month sampling period only fungal populations were significantly greater in the fertilized treatment (Table 4). Results for fungal populations grown on Martin's rose bengal medium were similar to DPY medium, so only DPY results are shown. Measurements of soil enzymes showed greater microbial activity in the fertilized soil than in the unfertilized soil (Table 5). Significant differences were measured for the enzymes acid phosphatase and β-glucosidase. Microbial biomass levels appeared higher in the fertilized soil than in non-fertilized soil during 11 of the 13 monthly sampling, but differences between the two treatments were not significant (Table 6). Concentrations of available N measured during the last five months of the study did not differ significantly between treatments. Ammonium increases ranged from 19 to 44 μg NH_4 N g^{-1} per week. The effect on N fertilization on microbial properties in the 7.5- to 30-cm soil zone was similar to that in the 0- to 7.5-cm zone. Microbial populations and activities were greater in the 0- to 7.5-cm zone than in the 7.5- to 30-cm zone and differences between the depths were statistically significant ($p < 0.10$) for all enzymes, fungal populations, actinomycete populations, and levels of available nitrogen (data not shown).

Higher populations and activities of soil microorganisms in fertilized soil as compared with

non-fertilized soil was due in part to increased growth of the corn crop as a result of fertilization. Greater crop yields result in greater crop residues, providing increased substrate for microbial growth. Increased residues will result in higher levels of soil organic matter which in turn contribute to better soil structure, infiltration, and soil water-holding capacity. Increased crop growth due to fertilization would increase root biomass and thus quantities of root exudates. Lynch and Panting (1980) noted that microbial biomass increases as root growth increases.

EFFECT OF GREEN MANURE ON SOIL MICROBIAL PROPERTIES

While N fertilization in the no-till treatments slightly increased microbial populations and activities, green-manuring with crimson clover had a much greater impact on soil microbial properties (Table 7). The soil into which the crimson clover had been incorporated contained significantly greater numbers of *Bacillus* spp., actinomycetes, and total bacteria when averaged across the year than did the soil from the N fertilized, conventionally tilled treatment which did not include clover. While populations of other groups of organisms appeared to be higher with the clover treatment, variability between replicates prevented the analyses of variance from detecting any other significant differences.

Microbial activity as measured by soil enzymes was significantly greater in the clover plots than the

non-clover plots (Table 5). Differences in enzyme activities between the two treatments were significant for alkaline phosphatase and β-glucosidase at $P < 0.05$, and for arylsulfatase at $P < 0.01$. Soil from the clover treatment had a significantly larger microbial biomass than did soil from the N fertilized, conventionally, tilled treatment without clover treatment (Table 6). In addition, when averaged across the 5 months measured, levels of available N were significantly higher in soil from the clover treatment ($P < 0.05$). Ammonium accumulation ranged from 29 to 58 μg NH_4-N g^{-1} soil per week.

While green-manuring with crimson clover had a significant effect on soil biological properties in the 0- to 7.5-cm zone, few significant differences were found between treatments in the 7.5- to 30-cm zone (data not shown). Microbial populations and activities were significantly greater in the surface soil than in soil at a depth of 7.5 - 30 cm. Differences between the depths were statistically significant ($p < 0.10$) for enzyme activities, fungal and actinomycete populations microbial biomass, and available N (data not shown). Although tests indicated a larger and more active microbial biomass in the clover treatment at the deeper depth, these differences were not statistically significant. When the crimson clover was plowed into the soil the bulk of the residue remained in the top 10 cm. Consequently, the soil microflora were concentrated in the zone with the greatest substrate.

The larger and more active microbial biomass in the surface soil from the green manure plots results from the green manure providing a source of C and N for microbial growth. Cropping systems which increase inputs of C through green manures, cropping sequences, or animal wastes have been shown to have more microbes and greater microbial activity than that found in systems which utilize fertilizer inputs (Bolton et al., 1985; Fraser et al., 1988; Buchanan, 1990). This organic material (C/N ratio of 17) had been plowed into the soil, providing intimate contact between organisms and substrate. Green-manuring increases soil organic matter levels leading to improved soil structure, infiltration, fertility, and water-holding capacity. The clover treatment had the highest soil moisture content of any treatment for 12 of the 13 months that samples were collected. The availability of a rapidly-decomposable substrate and a greater supply of soil water produced a better environment for microbial proliferation in the clover plots than in the no-clover plots.

SEASONAL FLUCTUATIONS IN THE SOIL MICROBIAL ECOSYSTEM

Soil microbial properties in all treatments varied significantly ($p < 0.05$) across the year (Tables 4, 5, 6, and 7). Enzyme activities increased in the spring, reaching a peak approximately in June (except for arylsulfatase which peaked in April) and then dipped, reaching a low level in August or September before reaching another peak in October or November. Actinomycetes and *Bacillus* spp. remained

relatively constant across the year while populations of the other organisms fluctuated in a manner similar to soil enzymes. Populations of microorganisms peaked in April, reached a low in August or September, and reached another peak in October or November. The minimal fluctuation of actinomycetes and *Bacillus* spp. is not surprising since actinomycetes produce somewhat drought resistant conidia and *Bacillus* spp. produce extremely drought resistant endospores which could initiate colonies when plated on a rich medium.

Microbial biomass peaked in May or June, stayed rather constant through October (although all non-clover plots showed a late summer dip) and then dropped again to the previous equilibrium level. These results agree with Gauger (1987) and Buchanan (1990). Bottner (1985) showed that drying the soil destroys a portion of the microbial biomass although previous levels were restored upon rewetting. The relatively constant biomass level in the clover plots during the dry period suggests that the organisms were dormant until water became more available. In the three non-clover treatments water was less available and some of the microbial biomass was probably destroyed during the drought period of August and September.

Several factors combine to explain seasonal fluctuations in soil microbial properties. As soil temperature increases in the spring the numbers and activities of soil microorganisms similarly increase. Also,

crop growth provides additional substrate for microbial proliferation. Lynch and Panting (1980) showed that microbial biomass increases with root growth as sloughed root cells and root exudates provide C for microbial growth. The growth of the clover in February and March, and corn starting in April help to explain the increase in microbial numbers and activities in the spring. The early spring growth of the crimson clover helps explain why levels of microbial biomass in the green manure plots began to increase before the microbial biomass levels of the other treatments.

Wardle and Parkinson (1990) suggest that microbial biomass dynamics and turnover are largely controlled by the dynamics of soil moisture. Soil moisture was significantly correlated with every soil biological property. A long dry period in August and September of 1990 dried the soil and caused a drop in enzyme activities and numbers of organisms. Between August 17 and October 5 a total of only 17 mm of rain fell. Consequently enzyme activities and microbial populations decreased during August and September. An increase in soil moisture in October caused enzyme activities and numbers of organisms to increase again. The fall peak noted for most microbial variables could be explained by the presence of adequate soil moisture, fresh crop residues after harvest, and decomposing root tissue providing a C source for microbial growth.

Although the management practices of fertilization and

green-manuring with crimson clover increased the populations, activities, and biomass of the soil microflora, the effect of the green manure was much more pronounced. The main influence of fertilization increased crop residues, root exudates and root biomass, providing additional substrate for soil microorganisms, and improving the environment for microbial growth. The incorporation of crimson clover residues also provided microbes with a source of C and N and the increased soil organic matter probably led to higher soil water-holding capacity. The effect of the green manure on soil biological properties was confined largely to the soil surface (0 - 7.5 cm).

Each microbial property varied significantly across the year. Microbial populations and activities increased in the spring reaching a peak approximately in May or June, dropped during the late summer, and climbed to another peak in October or November. These seasonal fluctuations seemed to be related to temperature, soil moisture, substrate availability, and root growth.

According to Doran and Werner (1990) sustained agricultural productivity may depend on the farmer's ability to select management practices which will enhance soil biological function in the fixation of atmospheric N and recycling of nutrients. This study suggested that reducing fertilizer inputs reduced both nutrient availability and the system's internal cycling efficiency. However, the practice of green-manuring with crimson clover represents an

alternative to inorganic fertilization and could optimize the system's ability to cycle and supply nutrients. While conventional cropping systems probably will produce greater grain yields than RCI systems utilizing leguminous green manures, the RCI system may be more economically and ecologically feasible. The RCI system optimizes the internal cycling of nutrients and maximizes the use-efficiency of external resources.

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Table 1. Soil physical and chemical properties of the soils studied.

Treatment	Block	Humic Matter[1] g/100 cm^3	Base Sat. (%)	Clay (5)	Sand (%)	Silt (%)	C.E.C. meq/100 cm^3	pH range during the study
No Fertilizer	1	0.4	81	23.8	61.7	14.5	6.2	6.0-7.0
	2	0.4	90	12.9	69.1	12.9	7.8	6.4-7.2
	3	0.6	84	17.7	66.7	15.6	7.7	6.1-6.8
	4	0.3	85	6.7	78.4	14.8	5.3	6.4-7.2
Fertilizer	1	0.4	77	26.4	54.9	18.7	6.9	5.5-6.6
	2	0.7	75	18.6	63.1	18.3	8.0	5.6-7.0
	3	0.5	82	15.4	68.6	16.0	6.8	5.5-6.6
	4	0.5	76	5.7	77.2	17.1	6.6	5.6-6.5
Clover	1	0.4	96	34.3	50.6	15.1	11.3	6.4-7.3
	2	0.3	87	32.6	50.3	17.1	9.2	6.2-7.0
	3	0.5	84	7.4	72.1	20.5	7.6	6.1-7.0
	4	0.5	93	11.2	69.5	19.3	11.2	6.4-6.9
No Clover	1	0.4	73	25.6	58.1	16.3	7.4	5.4-6.4
	2	0.6	71	8.7	76.0	15.3	5.5	5.4-6.4
	3	0.6	74	12.5	70.7	16.8	6.2	6.0-6.8
	4	0.3	79	6.6	81.1	12.3	5.8	6.0-6.9

[1] As determined by the North Carolina Department of Agriculture, Agronomic Division, Soil Testing Laboratory using an alkaline hydrolysis procedure.

Table 2. Description of the treatments studied.

Label	Tillage	Winter crop	Fertilizer	Pesticide[+]
			kg ha^{-1}	
No fertilizer	none	fallow	0	yes
Fertilizer	none	fallow	140	yes
Clover	Chisel plow/disk	crimson clover	no	no
No clover	Chisel plow/disk	fallow	140	no

[+] 7.4 L alachlor (Lasso) ha^{-1}, 5 L glyphosate (Roundup) ha^{-1}, and 5 L atrazine (AAtrex) at planting; 0.035 kg nicosulfuron (Accent) ha^{-1} postemergence; carbofuran (Furadan) was dropped with corn seed at 9 kg ha^{-1}.

Table 3. Effect of sampling position on some soil microbial properties.

SOIL MICROBIAL PROPERTY	CLOVER TREATMENT			FERTILIZER TREATMENT			SIGNIFICANCE	
	ROOT	SLOPE	INTER	ROOT	SLOPE	INTER	TREATMENT	POSITION
PLATE COUNTS	- - - - - - - log # of CFU g^{-1} $soil_{DW}$ - - - - - - -							
Bacillus	6.57	6.48	6.65	6.16	6.22	6.22	*[1]	NS
Fungi (DPY)	5.45	5.36	5.49	5.35	5.30	5.39	*	*
Fungi (MRB)	5.62	5.38	5.17	5.39	5.13	4.95	**	**
Pseudomonas	4.06	4.02	4.52	4.61	4.09	4.05	NS	NS
Actinomycetes	6.36	6.46	6.31	6.05	6.09	5.83	*	NS
Total bacteria	7.96	7.84	7.65	7.60	7.30	7.30	*	NS
Gram neg. bact.	6.82	6.91	7.44	6.93	6.41	6.39	NS	NS
SOIL ENZYME LEVELS	(μmols of p-nitrophenol g^{-1}_{DW} min^{-1}) X 10^{-2}							
Acid Phosphate	8.97	8.07	7.96	6.90	6.61	5.42	*	NS
Alkaline Phos.	1.45	0.71	1.20	0.72	0.06	0.10	*	NS
Arylsulfatase	1.29	1.01	1.00	0.70	0.51	0.48	*	NS
β-Glucosidase	3.02	1.85	2.08	1.18	1.01	0.87	*	NS
MICROBIAL BIOMASS - - - - - μg of microbial C g^{-1} $soil_{DW}$ - - - - - -								
	159.6	36.6	110.1	83.4	65.2	60.0	NS	NS
AVAILABLE NITROGEN - - - - - μg of NH_4^+ mineralized g^{-1} $soil_{DW}$ - - - -								
	73.7	45.6	67.9	40.3	16.6	19.8	*	NS

[1] *, ** indicates significance at probability levels $p < 0.05$, $p < 0.01$ respectively.

Table 4. Mean number colony-forming units (CFU) for different microorganisms from fertilized and nonfertilized soil (0 - 7.5 cm) sampled over a 13 month period.

Soil microbial property	JAN	FEB	MAR	APRIL	MAY	JUNE	JULY	AUG	SEPT	OCT	NOV	DEC	JAN	Ave for the year
	log # of CFUs g^{-1} $soil_{DW}$													
Bacillus spp.														
no fertilizer	5.68	5.47	5.46	5.62	5.61	5.66	5.55	5.76	5.44	5.81	5.27	5.59	5.36	5.56
fertilizer	5.82	5.51	5.59	5.66	5.70	5.78*[1]	5.52	5.72	5.53	5.90	5.70*	5.74	5.34	5.65
Fungi (DPY)														
no fertilizer	4.30	4.72	4.94	5.22	5.08	4.80	5.16	4.93	4.90	5.22	5.35	5.24	4.99	4.99
fertilizer	4.72	5.00	5.24	5.42	5.31	5.38†	5.28	5.26*	5.20*	5.45†	5.50	5.50*	5.26†	5.27*
Pseudomonas spp.														
no fertilizer	4.42	4.78	5.59	5.44	5.01	4.14	4.16	3.70	3.75	3.62	5.28	5.44	4.45	4.60
fertilizer	4.29	4.98	5.75	5.68	4.84	4.82	3.95	3.91	4.32	3.95	5.36	5.46	4.51	4.76
Actinomycetes														
no fertilizer	5.81	5.60	5.40	5.90	5.63	6.06	5.91	6.16	5.91	6.35	5.93	6.28	5.68	5.89
fertilizer	5.74	5.38	5.78	5.97	5.64	5.96	5.80	6.33	5.94	6.04	5.56	6.27	5.86	5.87
Total bacteria														
no fertilizer	7.49	6.98	7.18	7.44	7.24	6.89	7.25	7.00	7.24	7.52	7.33	7.60	7.53	7.28
fertilizer	7.53	6.87	7.33	7.55	7.49	7.02	7.20	7.00	7.25	7.48	7.50	7.64	7.84**	7.36
Gram negative bacteria														
no fertilizer	6.49	6.35	6.25	6.56	6.26	5.97	5.60	5.90	5.70	6.21	6.77	6.64	6.82	6.27
fertilizer	6.50	6.12	6.45	7.03	6.10	6.14†	5.45	5.84	5.96	6.30	6.88	6.77	6.96	6.35

[1] †, *, ** indicates significance at probability levels $p < 0.10$, $p < 0.05$, $p < 0.01$ respectively

Table 5. Mean extracellular enzymes activities in surface (0 - 7.5 cm) soil samples collected over a 13 month period.

Soil microbial property	JAN	FEB	MAR	APRIL	MAY	JUNE	JULY	AUG	SEPT	OCT	NOV	DEC	JAN	Ave for the year
	μmols of p-nitrophenol g^{-1} $soil_{DW}$ min^{-1} x 10^{-2}													
Acid Phosphatase														
no fertilizer	1.07	2.45	1.99	2.50	2.82	4.06	4.50	2.10	1.84	5.31	3.72	2.54	2.41	2.87
fertilizer	0.96	4.02*	3.24†	3.65	4.28*	7.06†	8.41	3.57	3.45	7.80	5.48	5.14*	5.08*	4.78*[1]
Alkaline Phos.														
no fertilizer	0.37	0.52	0.73	0.99	0.83	1.44	0.71	0.31	0.63	0.72	1.32	0.62	0.87	0.77
fertilizer	0.59	0.52	0.93	1.44	0.84	1.50	0.58	0.73	1.39	0.83	0.75	1.00†	1.02†	0.93
Arylsulfatase														
no fertilizer	0.36	0.69	0.62	0.68	0.45	0.62	0.45	0.53	0.29	0.61	0.74	0.61	0.49	0.55
fertilizer	0.46*	0.63	0.83	0.96	0.42	0.47	0.51	0.70	0.37	0.74	0.64	0.84†	0.64	0.63
β-Glucosidase														
no fertilizer	0.81	0.72	0.61	1.04	1.08	1.99	1.60	0.72	0.64	0.80	1.71	1.18	0.79	1.05
fertilizer	1.31*	0.97	1.14	1.53	1.46	2.65	2.71*	1.43*	1.23	1.41*	1.83	2.19*	1.56†	1.65*
Acid Phosphatase														
clover	1.31*	3.39	3.57	4.76	4.78	8.17	8.03	2.92	3.96	9.52	7.01	5.08	5.26	5.21
no clover	0.66	3.12	2.31	3.23	3.70	6.11	6.74	2.23	2.90	7.17	5.71	4.39	4.52	4.06
Alkaline Phos.														
clover	2.10*	2.42*	2.22*	3.33*	3.08†	3.43*	2.24*	2.12†	2.27	2.71*	3.46†	3.23*	3.95†	2.81*
no clover	0.36	0.53	0.87	1.09	0.78	0.62	0.27	0.46		0.59	1.03	0.77	1.12	0.71
Arylsulfatase														
clover	0.89**	1.15	1.26**	1.46*	0.98**	0.72	0.92**	1.10**	0.43*	1.28*	1.64*	1.28**	1.45†	1.12**
no clover	0.29	0.64	0.57	0.68	0.42	0.54	0.29	0.56	0.29	0.66	0.70	0.65	0.61	0.53
β-Glucosidase														
clover	2.67†	2.29*	1.95*	3.04*	2.74	3.56*	3.63*	2.88†	2.05*	2.91*	4.44†	3.63*	3.34*	3.01*
no clover	1.17	1.14	0.92	1.31	1.54	1.98	1.99	1.14	0.96	1.44	2.32	2.02	1.79	1.52

[1] †, *, ** indicates significance at probability levels $p < 0.10$, $p < 0.05$, $p < 0.01$.

Table 6. Microbial biomass C for surface (0 - 7.5 cm) soil samples collected over a 13 month period.

Soil microbial property	JAN	FEB	MAR	APRIL	MAY	JUNE	JULY	AUG	SEPT	OCT	NOV	DEC	JAN	Ave for the year
	μg of biomass C g^{-1} $soil_{DW}$													
Microbial biomass														
No fertilizer	78.0	78.6	68.3	59.8	48.9	144.4	93.6	63.7	68.8	81.3	64.8	41.7	40.5	71.7
fertilizer	38.4	78.8	94.0	96.1	56.8	180.2	122.3	68.7	95.4	100.3	55.8	52.3	66.8	85.1
Microbial biomass														
clover	88.2	84.0	121.6†	166.7†	182.3	188.0	165.2	151.0†	155.8†	150.7	113.6	71.1	99.5	133.7*
no clover	97.8	67.1	73.0	96.4	96.7	133.1	151.7	93.4	84.2	102.2	57.6	64.1	72.2	91.5

[1] †, *, ** indicates significance at probability levels $p < 0.10$, $p < 0.05$, $p < 0.01$ respectively.

Table 7. Mean number of colony-forming units (CFUs) for different microorganisms from surface soil of the clover and non-clover treatments soil (0 - 7.5 cm) sampled over a 13 month period.

Soil microbial property	JAN	FEB	MAR	APRIL	MAY	JUNE	JULY	AUG	SEPT	OCT	NOV	DEC	JAN	Ave for the year
	log # of CFUs g^{-1} $soil_{DW}$													
Bacillus spp.														
clover	6.30	6.55$^{\dagger 1}$	6.44	6.42	6.56†	6.61**	6.49*	6.46†	6.16†	6.77**	6.54*	6.75*	6.58†	6.51†
no clover	6.01	5.89	5.80	5.96	5.90	6.01	5.87	5.85	5.73	6.25	5.91	5.90	6.25	5.95
Fungi (DPY-5)														
clover	5.04	5.47	5.30	5.77*	5.76†	5.54	5.33	5.41	5.28	5.59	5.62	5.70	5.56	5.49
no clover	4.74	5.16	5.28	5.42	5.41	5.36	5.28	5.30	5.38	5.48	5.65	5.56	5.40	5.34
Pseudomonas														
clover	4.72	5.10	5.82	6.37†	5.24	4.72	4.01	3.90	4.28	4.01	5.41*	5.46	5.24	4.94
no clover	4.66	5.15	5.74	5.75	5.19	4.58	4.00	3.49	3.66	4.02	5.08	6.34	5.02	4.74
Actinomycetes														
clover	6.70	6.46†	6.46*	6.46*	6.45†	6.57*	6.62	6.42**	6.61	6.42**	6.67	6.59*	6.79**	5.59**
no clover	6.23	5.21	5.67	5.87	5.93	5.48	6.06	6.38	6.05	6.31	6.18	6.31	6.03	5.98
Total bacteria														
clover	7.93	7.52	7.72	8.65*	7.94†	7.65†	7.82†	7.78*	7.54†	8.08	7.88	8.11*	8.12	7.90†
no clover	7.51	7.48	7.13	7.84	7.48	7.22	7.63	7.35	7.23	7.71	7.84	7.84	8.01	7.56
Gram negative bacteria														
clover	7.00†	6.32	6.71	7.62	7.03†	6.42	5.76	6.11	5.92	6.84	7.16	6.98	7.20	6.70
no clover	6.63	6.82	6.28	6.99	6.59	6.10	5.92	5.88	5.63	6.59	7.24	6.86	7.17	6.52

[1] †, *, ** indicates significance at probability levels $p < 0.10$, $p < 0.05$, $p < 0.01$.