SOIL MICROBIAL BIOMASS AND NITROGEN MINERALIZATION INFLUENCED BY TILLAGE PRACTICES AND FERTILIZERS

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Abstract

Improved understanding of long-term tillage management on soil C and N dynamics is important for sustaining soil productivity. The objectives of this study were to determine effects of long-term tillage practices (9-yr) on seasonal and profile changes in soil microbial biomass C (SMBC) and N (SMBN), and mineralizable C and N in continuous cotton (*Grossypium hirsutum* L.) under no-tillage (NT), minimum tillage (MT) and conventional tillage (CN) under zero, low (20 lb N A^{-1}) and high (60 lb N A^{-1}) N fertilization. Additional measurements included soil bulk density and gravimetric water content. A Victoria clay (fine hyperthermic montmorillonitic Pellusterts) was sampled at three soil depths (0-2, 2-5 and 5-8 inch) before planting in March, during flowering in May, and after cotton harvest in August. No-tillage treatment tended to have higher soil bulk density and more surface water content with and without fertilization. Soil in the CN system showed the lowest water content. No-tillage also exhibited higher inorganic N concentration at planting than CN and MT. Inorganic N increased with the increase in fertilizer rates for all tillage systems but declined through the season. Microbial biomass C was greatest under MT at 0 to 8 inch depth as the season started because of residue accumulation and incorporation. No-tillage, however, had higher ability to maintain microbial biomass as season progressed and kept more biomass than CN and MT at flowering and harvest. The decrease in labial C quality and availability tended to decrease C and N mineralization potential through the season. Fertilization, however, caused increases in mineralizable C and N for all tillage treatments while NT with highest substrate showed greatest mineralization.

Introduction

Soil microbiological properties influence the soil N dynamics and plant availability of applied N. Previous research has suggested that microbial biomass and its activity is governed by soil properties and conditions like temperature, water content, C availability (Holmes and Zak, 1994), soil texture (Hassink, 1995), added N (Azam et al., 1988; Shen et al., 1984), and tillage (Matocha et al., 1988).

In southern Texas, conservation tillage (no-till, reduced till), which leave most or part of previous crop residues on the soil surface, is slowly increasing in popularity because of efforts to reduce soil erosion, to improve rainfall harvesting, to reduce costs, and to limit non-point pollution. It was reported that 22% of major crop (sorghum, corn, and cotton) land and 55% of small-grain land are under conservation tillage (Keith et al., 1994).

Several studies suggested that conservation tillage can maintain or increase soil organic C (Havlin et al., 1990; Tracy et al., 1990) which provides C source for microbial biomass accumulation. The C and N mineralization of those microbial biomass, however, can be reduced (Liu et al., 1995) because of impeded aeration (Baver et al., 1972), fined-texture (Hassink, 1995) and low temperature as a result of reducing heat input (Johnson and Lowery, 1985) in the reduce till soil. In contrast, conventional tillage with large amount of soil disturbance may cause more rapid mineralization process (Grace et al., 1993). In short-term, a rapid increase in the size of the microbial population and increased soil mineral N after conventional tillage were reported by Molope and Page (1986), and Carter and Rennie (1984). However, in many cases, short-term changes in microbial biomass does not reflect the tillage effects on nutrient supply for crop seasonal growth.

The level of added-fertilizers can influence the quantity of soil mineral N which may affect mineralization-immobilization turnover and its contribution to microbial biomass formation. In the experiments of Azam et al. (1988) and Grace et al. (1993), they reported the addition of fertilizer N significantly increased the total microbial biomass of the soil. In their measurement of the contribution of fertilizer N to the N mineralization, they found addition of fertilizer N not only increased the ammonium N from applied fertilizers but also stimulated the mineralization of soil native N. This phenomenon was called Added Nitrogen Interaction and defined as added N stand proxy for native N by Shen et al. (1984). The microbial biomass may act as a pool that stores the extra fertilizer N and then releases mineral N when crop is in a high N requirement.

In field situations, seasonal changes in soil water, soil temperature, soil structure and organic matter distribution and quality (Radke et al., 1985; Johnson and Lowery, 1985) may influence the N supply to crops through soil total microbial biomass accumulation and N mineralization process. More information is needed to identify the long-term effects of tillage practices and fertilizers on soil microbial biomass and N mineralization. The main purpose of this study was to investigate the effect of long-term tillage practices and fertilizer N on soil microbial biomass C and N and their mineralization potential within the growing season.

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Materials and Methods

Study Area and Soil

The experimental site was located at the Texas A&M University Agricultural Research and Extension Center Farm at Corpus Christi (28°N,97°30'W), which has a mean annual rainfall of 29.5 inch and a mean annual temperature of 22 °C. The field site was part of a long-term cotton (*Grossypium hirsutum* L.) experiment established in 1986. Cotton was rotated with corn every 4 yr under conventional tillage (CN), minimum tillage (MT) and no tillage (NT). Conventional tillage required 10 tillage operations and was compared with 5 operations for MT. In the NT treatment, fertilizer placement and planting were the only two operations which disturbed the soil. Cotton was delayed until early April due to wet fields and harvested in early August. Fertilizer sources were ammonium nitrate (AN, 33-0-0) and triple superphosphate (TSP, 0-46-0). The mixture of both materials was applied to cotton at preplant at three rates of (N-P_2O_5-K_2O) 0-0-0, 20-40-0 and 60-40-0.

The soil in the test site was Victoria clay (fine hyperthermic montmorillonitic Pellusterts) with 7.8 soil pH, 63% sand, 15% silt and 22% clay.

Treatments were arranged in a randomized complete block design with tillage as the main plot and fertilizer as the split plot with all treatments studied in four replicates.

Soil Sampling and Preparation

Soil samples were collected 2 days before fertilizer placement in March 1995, during flowering in May 1995, and two weeks after cotton harvest in August 1995. Four soil cores (1.7 inch diam) were taken randomly from planted row and between rows in each of nine split plots and all four replicate plots. Each core was divided into 0-2, 2-5 and 5-8 inch depth increments. The samples were ground and sieved (< 5 mm). All visible pieces of roots, residues and foreign materials were removed, and the fine materials were stored in a moist condition at 4°C until laboratory analyses were performed. The wet soil cores were air dried for 3 h prior to passing through a 5 mm-mesh sieve. Subsamples of each sample were dried at 80°C for 48 h for chemical analyses.

Microbial Biomass C and N

Carbon and nitrogen contents of the microbial biomass were determined by the chloroform fumigation-incubation method (Jenkinson and Powlson, 1976), as modified by Franzluebbers et al. (1994). Both fumigated and non-fumigated samples (30 g wet soil) were adjusted for a moisture content of 70% water-holding capacity prior to incubation. Soil microbial biomass C (SMBC) and N (SMBN) contents were calculated with the following formulas:

$$SMBC = (CO_2\text{-}C_{fumigated} - CO_2\text{-}C_{blank})/0.41$$

(Paul and Clark, 1989),

and

$$SMBN = (NH_4\text{-}N_{fumigated} - NH_4\text{-}N_{initial})/0.41$$

(Voroney and Paul, 1984).

Mineralizable C and N

Three unfumigated subsamples, each consisting of 30 g wet soil, were incubated in a 1-L air-tight canning jar in the presence of 10 mL of 0.5 M KOH and 10 mL of deionized water at 25 °C. The KOH solution was changed and the subsamples were removed at day 10, 20, and 30, taking into account the different rates of mineralization during the incubation.

Nitrogen mineralization was calculated by measuring the increase in NH_4-N and NO_3-N after incubation of soil samples. Concentrations of NH_4-N and NO_3-N of soil extracts were measured by Technicon AutoAnalyser (Blakemore et al., 1987) after extraction with 2 M KCl solution for 30 min using a soil-water ratio of 1:4.

Carbon mineralization was estimated by measuring the quantity of CO_2-C absorbed in KOH solution. At the sampling dates, accumulation of CO_2 was determined by titration of KOH excess with 1.0 M HCl and phenolphthalein after precipitation of the carbonates with an excess of $BaCl_2$ (Anderson, 1982). A control without soil was used for each incubation period to correct for atmospheric CO_2 and standardize the KOH.

Statistical Analysis

The significance of treatment effects was tested by analysis of variance (ANOVA). Sources of variation included tillage, N fertilizer, soil depth, sampling time and their interactions. Figures presented here are arithmetic means of the four replications. Differences among means were tested with a least significant difference (LSD) at $\alpha = 0.05$.

Results and Discussion

Soil Bulk Density and Water Content

Soil bulk density and water content were affected by tillage and fertilizer treatments (Fig. 1). No-till tended to have higher bulk density than CN and MT treatments at 0 to 8 inch depth, particularly in those plots with high fertilizers. Bulk density was significantly higher in high fertilizer soil at a depth of 5-8 inch for all treatments. Soil particle contraction, caused by fertilizer application, resulted in the increases in the sub-soil bulk density. Soil bulk density at this depth decreased in the order: NT > MT > CN, with NT significantly higher than MT and CN, and MT higher than CN. However, the tillage difference in bulk density between MT and CN appeared to be very small in soils receiving the higher fertilizer rate.

At surface soil (0-2 inch), the NT tended to be wetter than MT and CN. This higher level of surface soil moisture in

NT soil can possibly be explained by the mechanically disturbed soil in CN and MT having higher evaporative losses. Soil water content increased with soil depth for all treatments except for the MT which had some decrease in water content at 5-8 inch soil layer. Soil water in the deep soil layer (5-8 inch); however, was more stable for all tillages (slightly below field capacity) than that in surface soil.

Soil Inorganic Nitrogen

Following fall and winter mineralization, soil in the NT treatment exhibited high inorganic N at the 0 to 8-inch depth in the spring. Both CN and MT treatments showed significantly lower nitrate levels than the soil subjected to NT treatment. The inorganic N levels for the NT averaged from 23 to 45% higher than the CN treatment. Conventional tillage showed a nonsignificant increase in inorganic N over MT during the season.

At mid season or flowering, soil inorganic N levels showed a sharp decline in CN and NT treatments. There was considerable inorganic N (8.4 and 17.8 mg kg^{-1}) removed from CN and NT soils during the period between planting and flowering. The significant decline in the inorganic N of NT soil can be explained by the fact that the concentrated N in the soil surface was not incorporated and was vulnerable to loss through volatilization or denitrification (Sarrantonio and Scott, 1988), N immobilization by microbial biomass and N uptake by plants, while the N absorption by plants may be the major reason for the N decline in CN soil. The mid season inorganic N level under MT treatment remained almost unchanged from spring levels. The more residue (substrate) left in soil surface and the residue incorporation by the operations in MT practice could cause high activity of microorganisms (more attachment by microbial biomass), high net mineralization, and then redeem the N loss through environmental and plant uptake. Inorganic N concentrations continued to decline from flowering to harvest possibly due to microorganisms draw down during peak plant absorption of N (Matocha et al., 1988). Tillage effects became almost non existent at end of the season.

In spring, all tillage treatments exhibited an increase in the soil inorganic N with applied N fertilizers. Nitrogen increases were pronounced where high fertilizer was applied and may indicate rapid immobilization by the soil microbial biomass. Such immobilization seems to be possible when N fertilizer in excess of plant need is applied and is assimilated by the population of microorganisms. The presence of 437 to 4250 lb A^{-1} of cotton residues (Liu et al., 1995) with a low N content could account for immobilization of a portion of the N. The effect of fertilizers on soil inorganic N decreased in the order: NT > MT > CN. An application of 20 lb N A^{-1} resulted in 27, 16 and 15% higher soil inorganic N concentration than in the fertilizer control for NT, MT and CN, respectively. The inorganic N concentrations were 48, 47 and 26% higher for NT, MT and CN, respectively with an addition of 60 lb N A^{-1}. The fertilizer effects appeared to be reduced for all tillage treatments later at flowering and harvest possibly through plant uptake and N immobilization.

Microbial Biomass Carbon and Nitrogen

Soil microbial biomass vertical distribution was significantly altered by long-term tillage and fertilizer practices. Biomass-C levels (Fig. 2) in the soil surface layer under MT averaged 31% and 56% greater than under CN and NT treatments, respectively, for the three fertilizer N rates. The levels under CN and NT in the deeper soil (below 2 inch) averaged only 40 to 45% of those under MT. Greater biomass-C under MT indicated that a more uniform microbial biomass accumulation occurred within the 0 to 8 inch layer because of surface residue accumulation and less residue incorporation by minimized tillage operations. Biomass-C generally decreased with soil depth for the three tillage treatments. In the soil surface (0-2 inch) layers, biomass-C under MT averaged only 8% greater than those of the 2-5 inch layer and 35% greater than those of the 5-8 inch layer. However, the levels in the soil surface layer under CN and NT were approximately 84% and 129% of those found in the 2-5 inch layer and 31% and 119% of those in the 5-8 inch layer, respectively.

The vertical change in biomass-C was also influenced by fertilizer rates. At the 0 to 2 inch layer, biomass-C under CN and NT with fertilizer control was 2% and 24% smaller than with 60 lb N A^{-1}. In the 2 to 5 inch layer, however, biomass-C under CN and NT averaged 20% and 3% greater with 20 lb N A^{-1} and 197% and 21% greater with 60 lb N A^{-1}, respectively, as compared with fertilizer control. In the 5 to 8 inch layer, biomass-C under CN tillage at fertilizer rates of 20 and 60 lb N A^{-1} averaged 42% and 152% greater, respectively, than at fertilizer control. There was little or no difference in biomass-C due to applied N with MT at 0 to 8 inch depth, with the exception of 5 to 8 inch layer which resulted in a 35% decrease in biomass-C at a N rate of 20 lb A^{-1}. The increase in soil microbial biomass has been suggested as a result of immobilization of excess (fertilizer and released) N with incorporated residues (Thomsen, 1993 and Smith, 1994). However, the addition of the lower rate of N (20 lb A^{-1}) generally had little influence on the calculated biomass-C. Profile biomass-C showed little or no change from 20 lb N A^{-1} but a substantial change in the surface 2 inches with 60 lb N A^{-1}.

Seasonal patterns of biomass-C at 0 to 8 inch were similar in all treatments (Fig. 3), reaching maximum at planting in spring and declining through the season, with the exception of NT at 60 lb N A^{-1} treatment which had a increase in biomass-C at flowering. Biomass-C under MT without N fertilizer averaged 100% greater than under CN and NT at planting, and then showed a rapid decrease as was also observed with CN tillage from planting to flowering. At flowering, biomass-C under NT averaged 47% greater than under MT and CN but only 17% greater at harvest. An

application of 20 lb N A^{-1} slightly increased the biomass-C of MT, CN and NT at 21, 28 and 3%, respectively at flowering and 9, 23 and 28%, respectively at harvest. Soil microbial biomass C under MT, CN and NT was greater with 60 lb N A^{-1} than without N fertilization during all sampling periods, showing 1, 68 and 16% greater at planting, 27, 23 and 33% at flowering and 25, 22 and 25% at harvest. The rapid reductions in biomass-C in treatments with straw incorporation (MT, CN) as the season progressed from planting to flowering was also reported by Collins et al. (1992) and Holmes and Zak (1994) partly as a result of strong cooperative effects promoted by the presence of growing roots. Stimulation of microorganism activity by plant roots can influence microbial biomass C (Smith, 1994). While, the larger amount of surface plant residues may have provided available substrates for maintenance of microbial biomass in NT soil during the early growing season. After flowering, microbial biomass continued to decrease until the end of the season. There was no tillage effects on biomass-C at harvest, possibly because it was balanced by plant uptake and environmental loss.

The relationship between the C:N ratio of biomass-C and biomass-N in soil profiles was presented in Table 2. The microbial organisms in NT had lowest percentage of biomass-N at planting with a C:N ratio averaged 25% and 126% greater than MT and CN. The reduction in relative quantities of biomass-N under NT and MT treatments may be due to the large quantities of high C:N ratio prior season cotton residues left on the soil surface. In comparison, the larger percentage of high protein microorganisms in CN resulted in much lower C:N ratio. At each sampling time, C:N ratios increased at all soil depth under all tillage treatments. Averaged C:N ratios (over tillage) of microbial biomass at planting were 14 at 0-2 inch, 19 at 2-5 inch, and 25 at 5-8 inch layers. The increased biomass C:N ratio with soil depth could be attributed to the slower decomposition because of reduced substrate and poor aeration (Franzluebbers et al., 1994). This can also explain why the C:N ratio was highest in undisturbed soil of NT. A greater relative reduction in biomass-N, compared to biomass-C, as the season progressed because of greater N release for plant growth and a shift from more active to less active tissues (such as fungal to bacterial populations) (Collins et al., 1992) resulted in wider microbial biomass C:N ratio, averaged 19-42 and 20-41, at flowering and at harvest than at planting (12-26), but no significant differences were found during the period between flowering and harvest. These data suggested that turnover of the microbial biomass continued to supply nutrients to plants through the season.

Potentially Mineralizable C and N

Tillage and fertilizer management influenced potentially mineralizable C at a depth of 0 to 8 inch in similar seasonal pattern as potentially mineralizable N (Fig. 4). Without fertilizer, mineralizable C level under NT averaged 8% and 4% greater than those under MT and CN, respectively at planting, 8% and 13% greater at flowering, and then lower by 10% and 19% at harvest. The decline in mineralizable C through the season indicated a decrease in quantity and availability of labile C that were readily metabolized. For the soil received 20 lb N A^{-1}, there were significant increases in mineralizable C under NT sampled at all three sampling times and under MT and CN sampled only at flowering. About 441 mg C per kg soil was mineralized in 30 d incubation under NT at planting, up increased to 494 mg kg^{-1} at flowering and then decreased to 307 mg kg^{-1} at harvest. Similar patterns were also observed for MT and CN treatments. The NT resulted in higher mineralizable C than MT and CN because of the detention of active C in undisturbed soil, the difference becoming less marked as season proceeded. Mineralizable C increased in all tillage treatments as N rate was increased to 60 lb A^{-1}, with peak values at flowering showing 585, 488 and 469 mg kg^{-1} for NT, MT and CN, respectively. The differences in the N availability are likely to have contributed appreciably to the increase in mineralizable C after fertilization.

Mineralizable N was highest at planting for all tillage treatments in the N control. Although there was no tillage effects observed at planting and harvest, NT soil had mineralizable higher N levels at flowering which were 38% and 9% greater than MT and CN, respectively. Similarly to C mineralization, N mineralization was stimulated by N fertilization. Following application of 20 lb N A^{-1}, approximately 5, 10 and 5 mg more N per kg soil were released for NT, MT and CN at flowering and 10, 4, and 4 more at harvest, respectively, as compared to the N control. Differences due to fertilizer N at 60 lb A^{-1} were greatest at flowering (9, 13 and 9 mg more N released per kg soil) and harvest (12, 9 and 11 more). Differences in mineralizable N between flowering and harvest with N fertilization may be due to immobilization of N into the soil microbial biomass.

Potentially mineralizable N ranged from 28 to 12 mg N kg^{-1} in the 0 to 2 inch layer of nonfertilized NT soils and averaged 25 and 52% greater than that of MT and CN soils (Fig. 5). As was the case with microbial biomass, mineralizable N decreased with soil depth. The significant decline in mineralizable N observed in the 2 to 5 inch layer under NT and MT, could be partially due to the high soil bulk density. The application of fertilizer N at three tillage locations also resulted in significant increases in soil mineralizable N levels as compared with nonfertilizer treatments. The addition of 20 and 60 lb N A^{-1} increased mineralizable N levels in the surface soil layer by 4.2 and 10 mg N kg^{-1} for NT, 7 and 10 for MT, and 7 and 12 for CN, respectively. Nevertheless, the tillage effect on mineralizable N became nonsignificant at the 5 to 8 inch soil layer.

Conclusions

Soil bulk densities, microbial biomass C and N and mineralizable N were influenced the most in 0-5 inch soil layer. Seasonal changes and profile distributions of soil microbial biomass C and N and potentially mineralizable C and N were significantly influenced by tillage practices and fertilizer applications. Reduced tillage with and without fertilizers had greater seasonal changes in microbial biomass because of residue accumulation and incorporation. No tillage generally had slightly greater levels of microbial biomass after fertilization (60 lb N A^{-1}) with a large increase at flowering. Stratification of crop residues and organic matter and stimulation of bacterial population with N fertilization in the no tillage system is suggested as the mechanism for increased mineralizable C and N as compared to conventional tillage.

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Table 1. Soil inorganic N concentration as affected by tillage and fertilizer practices at a depth of 0 - 8 inch.

Tillage	Fertilizer (N lb A^{-1})	Sampling Time		
		Spring	Flowering	Harvesting
		---------- mg kg^{-1} ----------		
	0	18.2	19.0	10.7
MT	20	21.1	22.9	11.1
	60	26.8	25.6	13.1
	Avg	22.0	22.5	11.6
	0	21.6	13.0	8.7
CN	20	24.8	17.6	9.5
	60	27.2	17.6	11.6
	Avg	24.5	16.1	9.9
	0	26.6	16.0	10.6
NT	20	33.7	16.4	12.3
	60	39.4	13.7	14.7
	Avg	33.2	15.4	12.5
LSD (0.05)		4.36	5.41	1.03

Table 2. Microbial biomass C:N ratio distributions in soil as affected by tillage practices.

Tillage	Soil Depth (inch)	Sampling Time		
		Spring	Flowering	Harvesting
		---------- C:N ----------		
	0 - 2	15.2	25.1	22.8
MT	2 - 5	20.2	32.6	30.6
	5 - 8	26.0	42.0	40.7
	Avg	20.5	33.2	31.4
	0 - 2	7.9	12.8	13.9
CN	2 - 5	11.3	18.2	20.5
	5 - 8	16.4	26.5	24.1
	Avg	11.9	19.2	19.5
	0 - 2	18.7	30.1	28.7
NT	2 - 5	26.4	42.6	45.4
	5 - 8	32.2	52.0	49.8
	Avg	25.8	41.6	41.3
LSD (0.05)		3.2	5.1	7.98

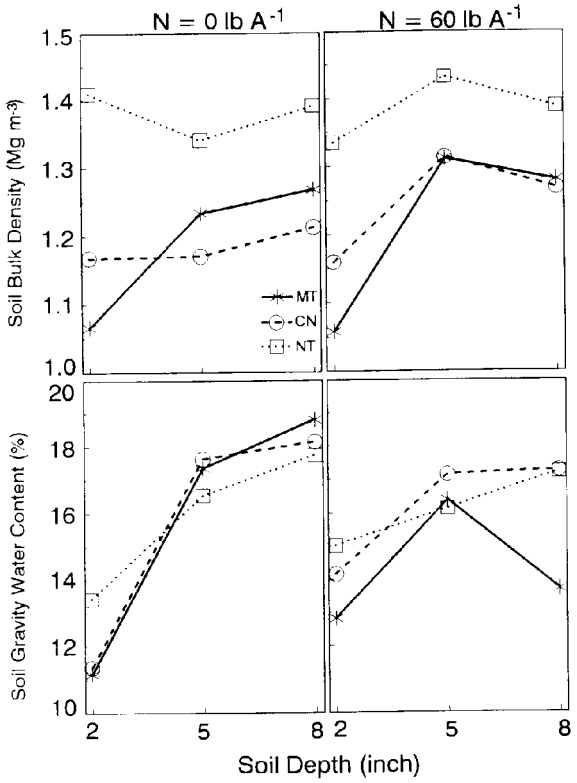

Fig. 1. Soil bulk density and gravity water content sampled in May as affected by tillage and fertilizer practices. LSD(0.05) to compare tillages within fertilizers at different depths for soil bulk density = 0.06, and LSD(0.05) to compare tillages within fertilizers at different depths for soil gravity water content = 1.83. CN – conventional tillage, MT = minimum tillage and NT = no tillage.

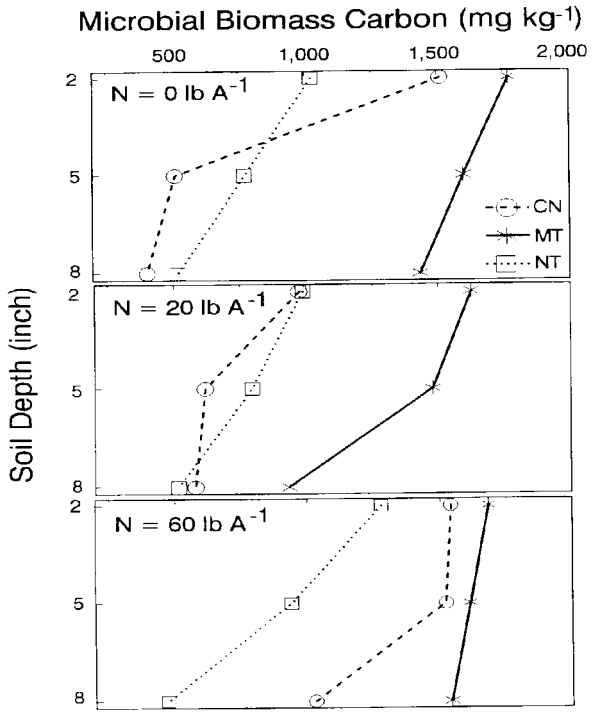

Fig. 2. Soil microbial biomass C distribution in soil profile at spring as affected by tillage practices and fertilizer rates. LSD(0.05) to compare tillages within fertilizers at different depths for microbial biomass C = 205. CN = conventional tillage, MT = minimum tillage and NT = no tillage.

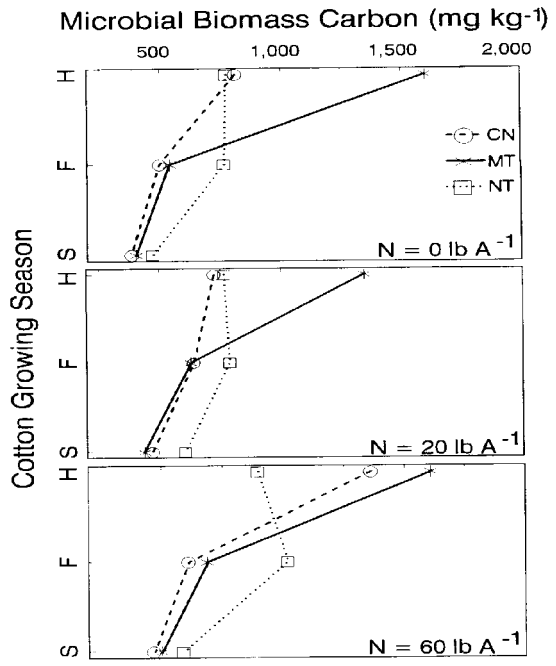

Fig. 3. Seasonal pattern of soil microbial biomass C at a depth of 0 to 8 inch as affected by tillage practices and fertilizer rates. LSD(0.05) to compare tillages within fertilizers at different sampling times for microbial biomass C = 224. Sampling times are spring (S), flowering (F) and harvest (H). CN = conventional tillage, MT = minimum tillage and NT = no tillage.

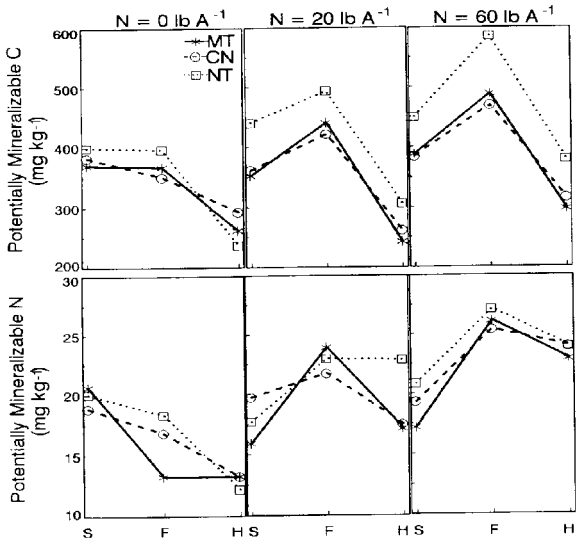

Fig. 4. Seasonal patterns of soil potentially mineralizable C and N at a depth of 0 to 8 inch as affected by tillage practices and fertilizer rates. LSD(0.05) values to compare tillages within fertilizers for different sampling times are 49.2 for mineralizable C and 2.94 for mineralizable N. Sampling times are spring (S), flowering (F) and harvest (H). CN = conventional tillage, MT = minimum tillage and NT = no tillage.

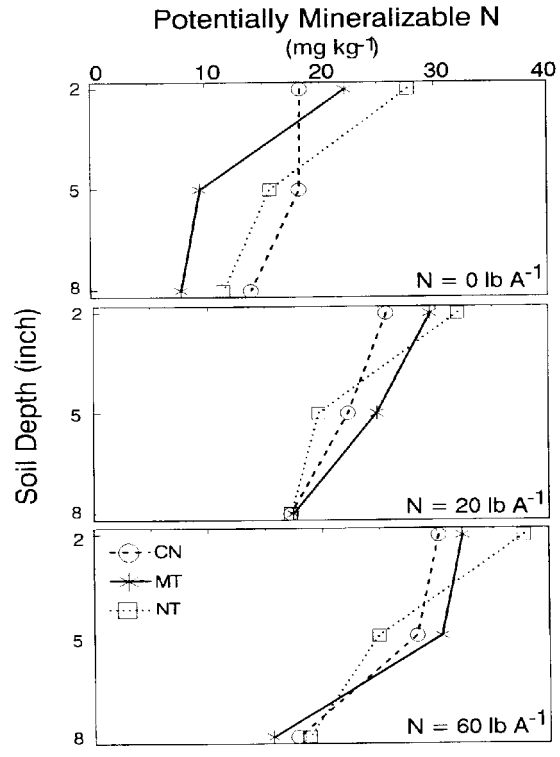

Fig. 5. Potentially mineralizable N distribution in soil profile at flowering as affected by tillage practices and fertilizer rates. LSD(0.05) to compare tillages within fertilizers at different depths for mineralizable N = 3.24. CN = conventional tillage, MT = minimum tillage and NT = no tillage.