Nitrification potential, dehydrogenase activity and microbial biomass in an argiudol soil cultivated with wheat under two tillering methods

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Abstract

The purpose of this work was to analyze the dynamics of soil biomass and its activity in a soil fertilized with N and cultivated under conventional or zero tillage systems. The soil under conventional tillage had larger biomass than under zero tillage but, in this latter condition, it was further increased by the N-fertilization. Dehydrogenase activity in the soil was identical under both management systems suggesting similar levels of activity. In addition, fertilization did not modify the nitrogen mineralization capacity of the soil. Only the addition of calcareous NH_4NO_3 , a fertilizer that releases nitrogen much faster than urea, resulted in the immobilization of nitrogen during wheat tillering, whereas urea did not alter soil N mineralization. The lack of a significant biomass response to tilling practices was reflected by the wheat biomass and grain yield, that was the same under both tilling systems. Only the total N content of wheat was higher under zero tillage than under conventional management, although this did not result in an increment in grain yield.

Keywords: microbial activity, autotrophic nitrification, cereals.

Resumen

Nitrificación potencial, actividad deshidrogenásica y biomasa microbiana en un suelo argiudol cultivado con trigo bajo dos sistemas de labranza

El objetivo de este trabajo fue analizar la dinámica de la biomasa microbiana y su actividad en un suelo fertilizado con distintas fuentes nitrogenadas y cultivado con trigo bajo labranza convencional y labranza mínima. En el suelo de las parcelas cultivadas bajo labranza convencional la biomasa microbiana fue mayor que en el suelo de las parcelas cultivadas bajo labranza mínima, provocando la fertilización nitrogenada un aumento de biomasa en este último. La actividad de deshidrogenasas fue similar en el suelo cultivado bajo los dos sistemas de labranza. La adición de fertilizantes nitrogenados no modificó la capacidad de mineralización del nitrógeno del suelo. Sólo la adición de NO₃NH₄ calcáreo, fertilizante de liberación más rápida que la urea, resultó en la inmovilización de nitrógeno durante el macollaje del trigo, mientras que la urea no alteró la mineralización del nitrógeno del suelo. La falta de un efecto significativo de las labranzas sobre la microflora del suelo se reflejó en el rendimiento de biomasa total y de grano del cultivo de trigo, que fue la misma bajo los dos sistemas de labranza. Sólo el contenido total de nitrógeno del trigo fue significativamente superior en las parcelas bajo labranza mínima, aunque esto no se reflejó en un aumento de la producción de grano.

Palabras clave: actividad microbiana, nitrificación autotrófica, cereales.

Introduction

The soil microflora is comprised of a complex group of microorganisms. One of its functions is to transform

those organic and inorganic substances found in the soils or incorporated to them. Therefore, microorganisms are responsible of the dynamics of nutrient flow in natural and cultivated ecosystems (Voroney and Paul, 1984). Although the microbial biomass represents only a small fraction of soil carbon (C) and nitrogen (N), it makes up the biological machinery of the mineralization of the organic material that enters the

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soil (Jenkinson and Rayner, 1977) and is, therefore, used as a predictive index of the changes occurring in organic matter content of the soil (Powlson *et al.*, 1987), and is a basic parameter of soil ecological studies (Joergensen, 1996).

Over the last few years in Argentina the use of conservationist tilling such as zero tillage have increased considerably (Crespo et al., 2001). Therefore, it is necessary to establish the effects of these management practices on the soil and also whether these techniques may lead to a sustainable use of this resource and to more economically viable production systems. Franzluebbers and Arshad (1997) found that tilling and soil texture changed not only the amount of particulate organic matter in the soils but also the mineralization induced by the activity of soil microflora. According to Aoyama et al. (2000), the proportion of C and N mineralized by the soil microflora was greater in macroaggregates than in microaggregates. Beare et al. (1992) observed, in soils managed under zero tillage system, that the mineralization activity of C was higher than in soils under conventional tillage systems. These results suggest that the microbial biomass metabolizes more easily the N and C present in the organic matter of the macroaggregates. Since tilling methods might alter the soil structure, they may indirectly affect the biomass and activity of soil microorganisms and with this the mineralization of C and N. Aon et al. (2001) reported that the potential mineralization of C and N was greater in soils under conventional tillage (CT) than under zero tillage (ZT) management systems.

The amount of residues on the soil surface can directly influence nutrient mineralization because the surface of contact between residues and microorganisms is reduced. Therefore, the microclimate of the soil surface under ZT is less favorable for soil decomposition than that of the soil under CT (Malhi et al., 2001). The impact of the presence of residues on N dynamics in the soil is illustrated by the results of Nyborg and Malhi (1989), who observed that the contents of NO_3^{-1} in the soils was greater under CT than under ZT, suggesting a higher activity of mineralization. Moreover, less N is immobilized in soils when the residues from the preceding crop are removed. Based on this, Malhi et al. (2001) suggested that a reduction in the mineralization capacity of nitrogen, a larger immobilization of N and a higher crop yield in soils under ZT might generate a N deficit.

The amount and activity of soil microorganisms could reflect the balance of available C and N in the

soils. Rovira and Davey (1974), and Burket and Dick (1998) suggested that the quantity of soil microorganisms and their activity are limited by the availability of carbon sources. However, Merckx *et al.* (1987) and Jingguo and Bakken (1997) showed that the availability of other mineral nutrient elements may limit Cmineralization in the soil.

Therefore, we hypothesized that the changes taking place in the amount and activity of the soil microorganisms might reflect the effects of the type of tillage and the amount of available N in the soil. Therefore, the aim of this work was: a) to analyze the biomass and activity of soil microorganisms in a wheat crop cultivated under two types of tillage systems: conventional and zero; b) to determine how N availability in the soil modifies the activity of its microflora.

Material and methods

The experiment was carried out in the Experimental Station Julio J. Hirschhorn, Facultad de Ciencias Agrarias y Forestales of the Universidad Nacional de La Plata, province of Buenos Aires (35° S), Argentina. The soil is a typical Argiudol with a silty franc texture, Serie Centeno fase por pendiente (Lanfranco and Carrizo, 1987), with mild internal drainage limitations. The experimental field had been managed under conventional tillage for three years previous to the experiment. This soil presented a pH of 5.86 (CaCl₂), an organic matter content of 47.9 g kg⁻¹, 43.6 mg kg⁻¹ of NO_3^- and a C/N ratio of 11.2. The crop was managed under two tillage systems: a) CT, consisting of two ploughs and two harrows at a depth of 15 cm and b) ZT, consisting of one tillage with a chisel tine. In both cultivation systems, sowing was done with a Deutz seed drill adaptable to each situation. In both tilling systems, two N fertilizer treatments were used at the time of sowing; one used commercial urea with 46% N and the other calcareous ammonium nitrate with 27% N, both at a ratio of 90 kg N ha⁻¹. Fertilizer application was performed at volley.

Before sowing, 4 l ha⁻¹ of glyphosate were applied as presowing total herbicide. Immediately after sowing, fertilizer was applied in the seed row with 100 kg ha⁻¹ of commercial calcium triple phosphate with 46% P_2O_5 . Wheat (*Triticum aestivum* L.) variety Buck Chambergo, was sown at a density of 120 kg of seed ha⁻¹.

Thirty days after sowing, plots were sampled using a borer of 9 cm diameter and 8 cm deep. A total of 6 subsamples were taken to make up the sample. Sampling was performed at three different times: sowing, tillering and anthesis.

Soil humidity and temperature were monitored along the experiment at two depths: 5 cm and 25 cm. Soil water content was determined by gravimetry and the temperature with a RDII Equidata equipped with a pT 100 thermoresistance.

Water content of the samples was reduced by sieving them through a 0.5 mm mesh and by keeping them at room temperature for 24 h. The microbial biomass (MB) was determined by the fumigation-incubation method (Jenkinson and Powlson, 1976). Amounts of soil corresponding to 50 g were weighed in triplicate and the water content was adjusted to 50% of the retention capacity. Two replicates per treatment were treated with chloroform vapors in a dessicator for 24 h at 28°C. The two control samples that were not fumigated were kept under the same conditions of temperature and humidity. Fumigated samples were reinoculated with 0.2 g fresh soil. Each sample was placed in an airtight container containing a test-tube with 15 ml 0.5 M NaOH to capture the CO₂ given off by the soil. The samples were incubated at 28°C for 10 days and at the end of the incubation period 3% BaCl₂ was added to each tube to provoke carbonate precipitation. Finally, the remaining alkali was estimated by titration with 0.5M HCl in the presence of phenopthalein.

Dehydrogenase activity was evaluated by colorimetric determination of tryphenil formazan (Casida *et al.*, 1964). Potential nitrification of the soils was determined by incubating 100 g soil samples, with the water content adjusted to 50% of the soil retention capacity, at 28°C for 3 weeks (Bloem *et al.*,1994). Two soil samples were supplemented with ammonium sulfate (10.6 g N in 100 g soil), while unamended soils were used as controls. At the end of the incubation period, 12.5 g soil were suspended in 20 ml 2 M KCl, and shaken for 2 h at 200 rpm. After filtration, the amount of nitrates was determined in an aliquot by the method of Cataldo *et al.* (1985). Soil nitrate determinations were done using a Nitracheck 404 portable reflectometer equipped with Merck 1020 reactive bands.

An outline was drawn up of the divided plots and its experimental design was a factorial of 2 tillages \times 3 fertilizer regimes \times 1 crop with 3 random repeats. The size of the plot was 3.5 m \times 10.40 m. Analysis of variance (ANOVA) was performed and significant differences were estimated by calculating the zero significant difference between means at a 5% probability level.

Results

Since microbial activity is modified by environmental factors, soil temperature and humidity were measured during the experiment at two different soil depths of 5 cm and 25 cm (Fig. 1). The water content of the soil cultivated under the two tilling systems was different. Throughout the experiment, the soil cultivated under ZT had a greater water content (approxi-

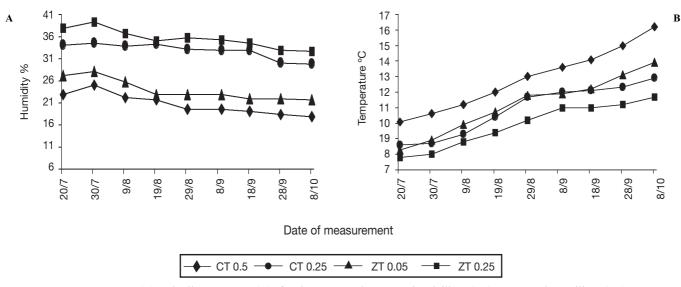


Figure 1. Water contents (A) and soil temperature (B) of a wheat crop under conventional tillage (CT) system and zero tillage (ZT) system.

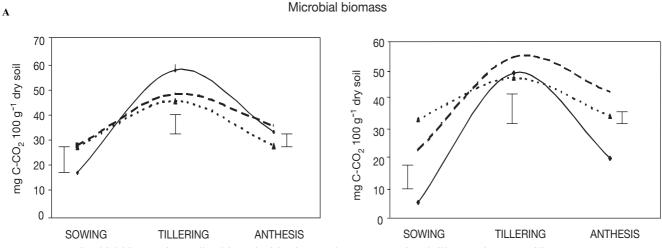


Figure 2. Microbial biomass in a soil cultivated with wheat under A) conventional tillage and B) zero tillage. Treatments: control soil (without fertilizer), -----soil + calcareous NH_4NO_3 , -----soil + urea. The total amount of N applied was 90 kg N ha⁻¹. Samplings were done at sowing time, tillering and anthesis. The bars in the figure represent the zero significant difference (MSD). The microbial biomass is expressed as mg of C-CO₂ released in 10 days by 100 g of dry soil.

mately 3-5%), wich was reflected at both depths (5 and 25 cm). The temperature in the soils increased over the experimental period as the environmental temperature increased. In soils cultivated under CT the temperature was around 2°C higher than in the soil under ZT and this difference was observed at both depths 5 cm and 25 cm. This difference remained during the entire crop cycle (Fig. 1).

The microbial biomass at the time of sowing was almost three times larger than in the soil under ZT, increasing at the time of tillering while decreasing during anthesis in both soils under CT and ZT (Fig. 2). The differences in the soil biomass under CT and ZT diminished with crop growth (Table 1). Fertilization with nitrogen compounds did not modify the effect of cultivation methods. Even more, it reduced the differences found in soil microbial biomass at the different sampling times (Fig. 2). The evolution of the microbial biomass in fertilized soils with any of the forms of N had the same profile along the crop cycle under both tillage systems although the absolute values were different. The biomass increased significantly during

Table 1. Microbial biomass

Cultivation	Sowing	Tillering	Anthesis	
СТ	16.74	57.99	33.04	
ZT	5.65	49.46	20.57	

The microbial biomass is expressed as mg of $C-CO_2$ released in 10 days by 100 g of dry soil. Determinations were made in a soil sample carrying out three repetitions per treatment. tillering and its evolution from sowing to anthesis appeared to be related to the rise in soil temperature (Fig. 1 and Fig. 2). In the plots fertilized with calcareous ammonium nitrate the size of the biomass, at tillering and anthesis, were higher than in those plots fertilized with urea, although these differences were not significant. It is interesting to note that in the soil under ZT the microbial biomass increased significantly during anthesis in response to nitrogen fertilization.

Figure 3 shows the values of microbial activity determined based on the reduction of 2-3-5 tryphenil tetrazolium chloride (TTC). The microorganisms activity at the time of sowing was similar in the soil under CT and ZT. At tillering, the microbial activity was lower than at sowing. Moreover, the activity was higher in soils tilled by CT rather than in soils tilled with ZT. At anthesis there was a considerable increase in dehydrogenase activity under ZT with values almost as high as those obtained during sowing. However, in spite of the differences observed in absolute activities, the evolution of the microbial activity in the soils under both cultivation systems was similar except at anthesis.

Nitrogen fertilization, as occurred with biomass, acted as a buffer of soil metabolic activity (Fig.3), revealing similar values at the different sampling stages. In soils fertilized with N there was a significant reduction in soil metabolic activity under the two cultivation regimes compared to the non fertilized control soil. At sowing time, the non fertilized soil had 58% more metabolic activity than the soil fertilized with N. At tillering, the activity of dehydrogenases in the non-

B

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Dehydrogenase activity

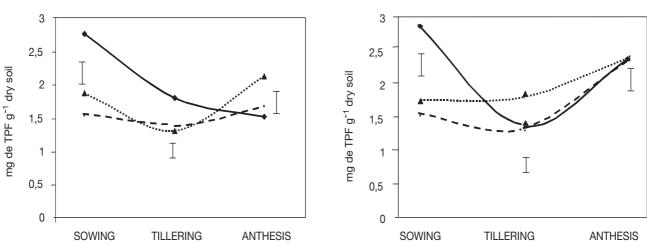


Figure 3. Dehydrogenase activity in a soil cultivated with wheat under a) conventional tillage and b) zero tillage. Treatments: control soil (without fertilizer),-----soil + calcareous NH_4NO_3 ,soil + urea. The total amount of N applied was 90 kg N ha⁻¹. Samples were taken during sowing, tillering and anthesis. Bars represent the least significant difference (LSD). Dehydrogenase activity was expressed as mg of tryphenil formazan (TPF) in 24 hours per g of dry soil.

fertilized soil was only 38% higher than the activity detected in the fertilized soil during anthesis.

In the soil under ZT the addition of N, also induced a significant reduction in activity at sowing. During tillering, the soil fertilized with urea presented a significant increase in the soil microbial activity and at anthesis no differences were detected between control soils and those fertilized with N. The nitrification potential observed at sowing, tillering and anthesis, under both tilling systems are presented in Fig. 4. The mineralization potential of N in the soil under CT was similar to that found in the soil under ZT. The correlation between biomass and nitrification potential was low and not significant in the soil cultivated with conventional tillering (r = 0.28 control; r = 0.29 soil fertilized with NH₄NO₃, r = 0.19 soil

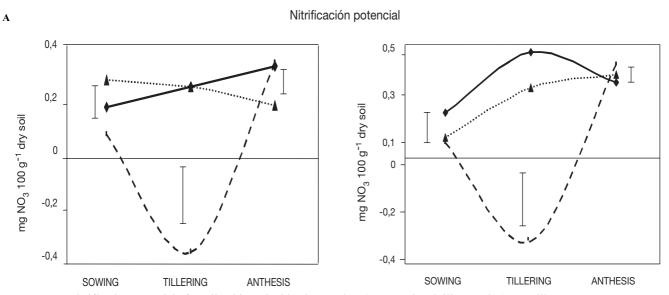


Figure 4. Nitrification potential of a soil cultivated with wheat under A) conventional tillage and B) zero tillage. Treatments: $_$ control soil (without fertilizer),-----soil + calcareous NH₄NO₃, ..., soil + urea. The amount of N applied was 90 kg N ha⁻¹. Samples were taken during sowing, tillering and anthesis. Bars represent the least significant difference (LSD). The nitrification potential is expressed as mg of NO₃⁻ per 100 g of dry soil.

fertilized with urea). In contrast, the correlation was significant in the soil cultivated under zero tillage and supplemented with calcareous NH₄NO₃ and in the unfertilized soil, with values of r = 0.39 and 0.84, respectively. The soils of the wheat crop under ZT or CT fertilized with urea revealed potential nitrification values similar to those of the unfertilized crop (Fig. 4). In contrast, in the soil fertilized with calcareous NH₄NO₃ the values of nitrification potential were negative, indicating that the ammonia added to the soil was immobilized due to a N deficiency at tillering (Fig. 4). Moreover, this occurred in the soil fertilized with calcareous NH₄NO₃ both under CT and under ZT, which confirms the effect of adding a nitrogen fertilizer with a rapid rate of N-release. At anthesis, the potential nitrification levels were similar to those found during sowing in the soil with and without N addition.

In Table 2 it can be observed that the cultivation systems did not alter the yield of the wheat crop, since wheat grain yield and total biomass under CT and ZT were similar. Only the total N content of the plant was significantly different for the different tilling systems, being the total N content under ZT significantly higher than under CT.

As expected, N fertilization provoked significant increases in the total N content of the plants and in dry matter and grain yield, compared to the control unfertilized plants. However, no significant differences in any of the parameters mentioned were found between the two fertilizers used. NO_3^- content in soils (Table 3) was greater at tillering than at sowing due to the ac-

Table 2. Total N contents in the plant, shoot biomass and grain yield of wheat plants cultivated under two tilling systems

Treatment	Total content of N (hg ha ⁻¹)	Aerial biomass (hg ha ⁻¹)	Grain yield (hg ha ⁻¹)
Tillering			
СТ	195 a	15,030 b	6,210 a
ZT	216 b	16,590 b	6,150 a
Fertilization			
CAN	208 a	15,500 a	6,240 a
Urea	225 a	16,900 a	6,670 a
Test	161 b	13,310 b	5,290 b

Equal letters indicate non significant differences. Different letters indicate significant differences at the 5% level. CT= conventional tillage. ZT= zero tillage. TEST = unfertilized soil. Urea= soil fertilized with urea (90 kg N ha⁻¹). CAN = soil fertilized with calcareous ammonium nitrate (90 kg N ha⁻¹).

Table 3	$. N-NO_{3}$	contents	of th	e soil
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	CT Tillering	CT Anthesis	ZT Tillering	ZT Anthesis
CAN	80	14	75	20
Urea	90	50	80	29
Test	12	12	11	16

The N-NO₃ content of the soil was expressed as mg kg⁻¹ of NO₃. Determinations were made in a soil sample, carrying out three repetitions per treatment.

tion of nitrogen fertilizers, and was reduced at anthesis. The lowest level of NO_3^- was recorded in soils under CT. This could be attributed to the presence of organic wastes in ZT, contributing this to the input of NO_3^- to the soil.

Discussion

The activity of the microorganisms that make up the soil microflora is responsible for the dynamic of the nutrients in the soil (Voroney and Paul, 1984). The physical and chemical changes provoked by tilling in the soils can alter not only the amount but also the type of microorganisms present in the soil. This work confirms previous findings by Balatti and Diosma (1997) that cultivation techniques do not alter the microbial biomass over short periods of time and in the crop conditions described, at least based on the analytical approaches used. Franzluebber and Arshad (1997) found that cultivation techniques altered the amount of particulate organic matter in a soil only after long periods of time. The changes in biomass observed at the different stages of crops growth were probably buffered by N additions, revealing that N was probably limiting microbial development in the soil analyzed. Soderstrom et al. (1983) demonstrated that the microbial biomass could be smaller in soils fertilized with N since the C/N ratio is reduced. Other authors (Merckx et al., 1987) have suggested that not only the availability of C, but also that of other soil elements, regulate soil microorganism dynamics. Jingguo and Bakken (1997) demonstrated that microbial biomass of soils with a plant cover is smaller than that of bare soils and associated this with the plants competition with microorganisms for N. In this experiment, biomass values were low at sowing and increased at tillering, probably due to the increase in C generated by the organic material supplied by the plants. However, the size of

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the microbial biomass was reduced at anthesis probably because the crop had a higher N demand. This was supported by the NO₃⁻ values measured in the soils (Table 3). The lowest NO_3^- values were observed in the control soil not fertilized at tillering, coinciding with a high value of both microbial biomass and N absorption by the plant. In soils fertilized with urea and calcareous NH₄NO₃, the level of NO₃⁻ is similar under CT and ZT during tillering. At anthesis, the soil under CT fertilized with urea presented higher levels of NO₃than soils fertilized with calcareous NH₄NO₃. This could be either the result of using urea, a fertilizer that releases nitrogen at a lower rate or that the NO3⁻ provided by calcareous NH₄NO₃ was less stable in the soil, being washed out by the rainfall. A similar behavior was observed in soils cultivated with ZT, but the differences were smaller. The size of the biomass of soils amended with N suggested that nitrogen fertilization only resulted in reductions of the changes observed among soil tilling systems.

The addition of nitrogen fertilizers that release NO₃⁻ at different rates did not alter the biomass dynamics. In accordance with these results, Videla *et al.* (1996), Ferrari *et al.* (1997) and Henriksen and Breland (1999) found that biomass was the only parameter that did not reflect the changes induced by nitrogen fertilization in the mineralization of organic matter. However, Beare *et al.* (1992) proposed that fumigation with chloroform was not the most suitable approach to use when the soils had an important contribution from organic wastes as occurs in field crops under zero tillage. Therefore, factors other than N were probably responsible of the evolution of the soil biomass such as the availability of phosphorus or other nutrient elements and also the methodology used.

The activity of dehydrogenases is a measure of the biological activity of the soil microorganisms (Beyer *et al.*, 1993). However, the amount of microorganisms in the soil is not always associated with a greater metabolic activity (Diosma and Balatti, 1998). In these experiments, the activity of dehydrogenases was similar in soils under different tilling systems. This latter observation is interesting since Aon *et al.* (2001) found that in soils with a short agricultural history, the mineralization potential of organic matter and nitrification is greater under CT. However, Beyer *et al.* (1993) and Burket and Dick (1998) found that the activity of dehydrogenases depends more on the type of soil than on the management practices used. Microbial activity of the soil amended with N fertilizers was significantly

lower than that of non-fertilized soil at sowing, even though the rate of increase in biomass in these two soils was similar. Later, at tillering and anthesis, the differences in dehydrogenase activity become smaller and disappeared. In other words, in nonfertilized soil the metabolic activity at sowing tends to be high, and decreases along crop growth. This suggests that the crop may be competing with the microorganisms for some substrate or element in the soil. In soils fertilized with N the initial value of the C/N ratio alters the metabolic activity of microorganisms, though later the ratio remains constant over the rest of the crop cycle. Beyer et al. (1993) and Lovell and Hatch (1998) reported an increase in dehydrogenase activity in response to nitrogen fertilization. In contrast, Burket and Dick (1998) reported that in soils fertilized with N the metabolic activity, determined by the activity of certain enzymes, tended to decrease. They suggested that the addition of N to the soils that already have a good supply of nitrogen leads to the condensation of N-rich elements. Our results suggest that an effect of this type might have taken place, since the experiment was conducted in a high organic matter content and therefore, the addition of N could have lead to a similar situation as that described by Burket and Dick (1998).

The mineralization capacity of N in the soil under study was not affected by the cultivation techniques used. Diosma and Balatti (1998) reported that the number of ammonia oxidizing microorganisms was similar in soils cultivated with CT and two conservationist tillages. The rise in microbial biomass did not modify the mineralization activity of N, probably due to the high content of N in the soil. Falotico et al. (1999) found that the interaction between tilling and nitrogen fertilization was not significant. The nitrification potential of the soil was similar under both cultivation techniques and increased along wheat cycle by approximately 80-100% of the value recorded at sowing. Only the formulation of the fertilizer applied modified the soil's mineralization capacity. The potential nitrification values observed suggest that nitrogen fertilizer that releases N at a fast rate, such as calcareous NH₄NO₃ are unsuitable to satisfy the soil's N demand. An evidence of this is the immobilization of the amended N that occurred at tillering. However, the values of NO_3^- present in the soils suggest that the availability of NO₃⁻ was the same whether fertilized with urea or with calcareous NH₄ NO₃ (Table 4). Several recent studies have been aimed at analyzing the effect of calcareous NH₄NO₃ additions.

Videla et al. (1996) observed a non-significant microbial immobilization in fertilized soils. In this work, it has been found that except at tillering the N-mineralization capacity of the soil was correlated with the size of the microbial biomass. The decrease in N mineralization in the crop occurred concomitantly with a reduction in dehydrogenase activity. Since nitrification was similar between sampling periods and tilling treatments, it is clear that C was not a limiting factor since neither crop development nor the presence of a larger amount of residues in the soil under ZT modified the soil capacity to mineralize N. In contrast, Nyborg and Malhi (1989) found that N mineralization was lower under ZT due, among other factors, to the immobilization of N in the soil by the organic residues. These differences were probably due, either to the amount of organic material present in the soil used by Nyborg and Malhi that was much greater than the organic matter contents of the soils used in this experiment, or to the low N content at the beginning of the experiment.

The analysis of biomass, its activity and potential nitrification suggest that conservationist tillages, does not provoke, at least in the short term, significant changes in the soil microbial activity compared to conventional tillage systems, in the soil and climate conditions found in the depression of the River Salado. This was also reflected in the crop yields since no significant differences were observed either in the yields of the aerial biomass or grain, due to tilling system. Only the amount of total N was higher in those wheat plants cultivated under ZT, possibly due to the high organic residue content of the soil.

Fertilization, as expected, has generated increases in biomass, in grain yields and in total N content compared to the unamended control. Although the application of two types of fertilizers modified soil nitrification, it did not alter the response of the wheat crop to N addition.

Soil tilling using conservationist techniques did not produce any significant changes in the activity of the soil microorganisms studied detectable early on. Furthermore, nitrogen fertilizers that differ in the rate of NO₃ release, can not only alter the potential nitrification capacity of the soils but also buffered those changes in microbial biomass and activity that are associated with the tilling system used and the sampling time along crop growth cycle. Mineralization of C in the soils studied was not limited by N availability.

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