CHANGES IN SOIL MICROBIAL ACTIVITY FOLLOWING CONSERVATION TILLAGE PRACTICES IN A *SORGHUM* FIELD UNDER SUBTROPICAL CONDITIONS

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Abstract
A field experiment was carried out in a degraded sorghum field, under warm subtropical conditions, to determine the influence of conservation tillage practices such as no tillage and reduced tillage (subsoil-bedding and shred-bedding), and conventional tillage practices such as mouldboard ploughing, on physical and microbiological soil quality indicators, over a period of three years. An adjacent soil under natural vegetation was used as a standard of local high quality soil. Conservation tillage systems, in particular no tillage, increased crop residue accumulation on the soil surface. Organic matter decreased with increasing tillage at all depths and was 33% greater with no tillage compared with the average of the other tillage treatments in the surface 0 to 5 cm. No tillage produced values of soil organic matter, available P and nitrate similar to soil under natural vegetation. The no tilled soil had values of water soluble C, dehydrogenase, urease, protease, phosphatase and β-glucosidase activities, and aggregate stability higher than tilled soils but lower than the soil under natural vegetation. The enzyme activities and aggregate stability reflected early changes in soil the profile to a greater extent than did physical-chemical and chemical properties. The no tillage system was the most effective for improving soil physical and biochemical quality. In the medium-term, this conservation tillage system is still far from achieving the quality levels of soil under natural vegetation.

Additional Keywords: aggregate stability, microbial activity, crop residue, soil enzyme activities

Introduction
Conservation tillage systems in Mexico were implemented by growers in the late 1970s with the goal of promoting long-term sustainability of agricultural ecosystems. Conservation agriculture refers to several practices which are based on the use and management of prior crop residues for covering at least 30% of the soil surface, altering soil composition, structure and natural biodiversity as little as possible and defending it from erosion and degradation.

There is a need to select soil properties that rapidly respond to changes in soil quality while a particular conservation practice is being carried out in order to ascertain whether that practice is recommendable or not (Roldán *et al*., 2003). Soil enzyme activities have been suggested as potential indicators of soil quality and productivity because of their essential role in soil biology and in the cycling of the most important nutrients, ease of measurement and rapid response to changes in soil management (Kandeler *et al*., 1999). However, no information is available on changes in enzyme activities following tillage practices in subtropical agroecosystems.

The objective of this study was to determine the influence of conservation tillage practices, such as no tillage and reduced tillage (subsoil-bedding and shred-bedding), on physical and microbiological soil quality indicators in a degraded sorghum field under warm subtropical conditions. An adjacent soil under natural vegetation was used as a standard of local high quality soil.

Materials and Methods
Site description
This research was conducted at the Río Bravo experimental site, in Northern Tamaulipas, Mexico (25º 57'N, 98º01'W). The dominant soil type is Vertisol developed from alluvial sediments with a clay texture (28% sand, 41% clay and 31% silt), containing 1.2% organic matter and with a pH of 7.8 (1:2 soil:water). The climate of the region is classified as warm subtropical, with hot, wet summers and dry winters. The annual temperature averages 23ºC and the annual rainfall averages 635 mm. The topography of the area is mainly flat and slopes do not exceed 3%. The climax vegetation of this area has almost disappeared due to agriculture, which is currently represented by shrub species, such as *Prosopis juliflora* and *Acacia farnesiana*, and halophytic pasture.

Experimental design and layout

The experiment was conducted using a completely randomised block design with three field replications for each treatment. Plots measured 22.4 by 52 m. The tillage treatments examined were mouldboard plough (disking stalks after harvest, followed by mouldboard plough and disking, then building the rows); subsoil-bedding (shredding stalks after harvest, followed by subsoiling on row centres and forming beds in the same operation); shred-bedding (shredding stalks after harvest, followed by bedding on the old rows); and no tillage (shredding stalks after harvest and spraying glyphosate [1.5 l ha^{-1}] and 2-4 D amine [1.5 l ha^{-1}] as needed for weed control). *Sorghum bicolor* (L.) Moench was planted in early February and harvested in the first half of June each year from 2001 to 2003.

Chemical, biological, biochemical and physical analyses

In soil aqueous extracts, water soluble carbon was determined by wet oxidation with $K_2Cr_2O_7$ and measurement of the absorbance at 590 nm (Sims and Haby, 1971). Dehydrogenase activity was determined according to García *et al*. (1997). For this, 1 g of soil at 60% of its field capacity was exposed to 0.2 ml of 0.4% INT (2-*p*-iodophenyl-3-*p*-nitrophenyl-5-phenyltetrazolium chloride) in distilled water for 20 h at 22ºC in darkness. The INTF (iodo-nitrotetrazolium formazan) formed was extracted with 10 ml of methanol by shaking vigorously for 1 min and filtration through a Whatman N° 5 filter paper. INTF was measured spectrophotometrically at 490 nm. Urease and N-α-benzoyl-L-argininamide (BAA) hydrolyzing protease activities were determined by measuring the NH_4^+ released in the hydrolysis reaction after addition of urea and BAA, respectively (Nannipieri *et al*., 1980). Phosphatase and β-glucosidase activities were determined using as substrates *p*-nitrophenyl phosphate disodium (PNPP, 0.115 M) and p-nitrophenyl-β-D-glucopyranoside (PNG, 0.05 M), respectively. Two millilitres of 0.5M sodium acetate buffer at pH 5.5 and of 0.1 M maleate buffer at pH 6.5, respectively and 0.5 ml of substrate were added to 0.5 g of soil and incubated at 37°C for 90 min. The reaction of phosphatase activity was stopped by cooling at 2°C for 15 min. Then, 0.5 ml of 0.5 M $CaCl_2$ and 2 ml of 0.5 M NaOH were added, and the mixture was centrifuged at 4000 rpm for 5 min. To stop the reaction of β-glucosidase activity, tris-hydroxymethyl aminomethano was used according to Tabatabai (1982). The *p*-nitrophenol (PNP) formed was determined in a spectrophotometer at 398 nm. The percentage of stable aggregates was determined by the method described by Lax et al. (1994). A 4 g aliquot of sieved (0.2-4 mm) soil was placed on a small 0.250 mm sieve and wetted by spray. After 15 min the soil was subjected to an artificial rainfall of 150 ml with an energy of 270 Jm^{-2}. The remaining soil on the sieve was put in a previously weighed capsule (T), dried at 105ºC and weighed (1). Then, the soil was soaked in distilled water and, after 2 h, passed through the same 0.250 mm sieve with the assistance of a small stick to break the remaining aggregates. The residue remaining on the sieve, which was made up of plant debris and sand particles, was dried at 105ºC and weighed (P2). The percentage of stable aggregates with regard to the total aggregates was calculated by (P1-P2)x100/(4-P2+T).

Statistical analysis

Treatment effects on measured variables were tested by analysis of variance, and comparisons among treatment means were made using a least significant difference (LSD) test calculated at $P<0.05$. Statistical procedures were carried out with the software package Statgraphics for Windows 7.0.

Results and Discussion

Tillage intensity decreased water soluble C (Table 1). The water soluble C content of the soil under mouldboard was on average about 20% less with respect to no tillage soil. Water soluble C was affected significantly by soil depth. The labile organic C fractions are used by the soil microbial community as an energy source for metabolic activity. The study of these fractions is important in agricultural soils, since they determine soil microbial activity and perform a structural function. On the basis of the water soluble C data, we can suppose, therefore, that all tilled soils, and even no tilled soil, had less microbial activity than the soil under natural vegetation, at all depths.

Dehydrogenase activity was decreased significantly by agricultural use of soil, in particular by intensive (mouldboard) and reduced tillage (subsoil-bedding and shred-bedding), as shown in Table 1. These differences in dehydrogenase activity, between no tilled and tilled soils, decreased with soil depth. Dehydrogenase is an oxidoreductase, which is only present in viable cells. This enzyme has been considered as a sensitive indicator of soil quality and it has been proposed as a valid biomarker to indicate changes in total microbial activity due to changes in soil management, under different agronomic practices and climates. Dehydrogenase activity responded to the treatments in a similar manner to the water soluble C fraction, i.e. increasing with the adoption of no tillage, in direct proportion to the accumulation of crop residues in the soil surface. The decomposition of such residues in soil releases essential nutrients, such as N, P and S, required for both plant and microbial growth. Only in the

surface layer (0-5 cm) was dehydrogenase activity in the no tillage soil significantly lower than in the soil under natural vegetation.

Measurement of soil hydrolases provides an early indication of changes in soil fertility, since they are related to the mineralisation of important nutrient elements such as N, P and C. The use of a single enzyme has been criticised by several authors, mainly because enzyme activities catalyse specific reactions and they are substrate-specific (Jimenez et al., 2002). With few exceptions, tillage had negative effects on the hydrolase activities considered in this study (urease, protease-BAA, phosphatase and β-glucosidase), at all soil depths, mainly with the adoption of mouldboard (Table 1). Soil management influences soil microorganisms and soil microbial processes through changes in the quantity and quality of plant residues in the soil profile (Kandeler *et al*., 1999). In conventionally cultivated soils, organic matter is more thoroughly distributed than in reduced tillage soils, where crop residues are concentrated on the soil surface (Salinas-García *et al*., 2002). As a consequence, microbial activities were more evenly distributed throughout the plough layer in the tilled systems, except for protease activity. However, there was no clear relationship between microbial activities and soil depth in the no tillage soil. Only the activity of protease, an enzyme involved in N cycling, increased significantly ($p<0.001$) with depth in all the treatments. The lack of a depth effect on acid phosphatase activity, which is involved in P cycling, is most likely the result of this enzyme predominantly being secreted by plant roots (and associated mycorrhiza and other fungi). No tillage produced lower hydrolase activities, to a depth of 10 cm, than in the soil under natural vegetation.

Table 1. Labile organic matter fraction and biochemical properties of the soil under natural vegetation and different management tillage systems (n=3).

	Soil depth (cm)								
	0-5	**5-10**	**10-20**	**0-5**	**5-10**	**10-20**	**0-5**	**5-10**	**10-20**
	Water soluble C (μg g^{-1})			**Dehydrogenase (μg INTF g^{-1})**			**Urease (μmol NH_3 $g^{-1}h^{-1}$)**		
Natural vegetation	187a*	199a	173a	100.2a	68.9a	40.9a	1.93a	2.18a	2.15a
Mouldboard	94c	90c	96b	45.2c	44.6c	44.3a	0.63c	0.65c	0.72b
Subsoil-bedding	106bc	115b	98b	50.0c	56.4abc	51.1a	0.62c	0.77bc	0.84b
Shred-bedding	105bc	115b	105b	48.5c	51.7bc	49.9a	0.84bc	0.70bc	0.81b
No tillage	113b	118b	104b	66.1b	57.7ab	40.8a	1.25ab	0.83b	0.90b
	Protease (μmol NH_3 $g^{-1}h^{-1}$)			**Phosphatase (μmolPNP$g^{-1}h^{-1}$)**			**β-glucosidase (μmolPNP$g^{-1}h^{-1}$)**		
Natural vegetation	2.05a	2.34a	1.25a	1.18a	1.03a	0.74a	1.47a	0.85a	0.31ab
Mouldboard	0.23d	0.31c	0.43b	0.29c	0.30c	0.29c	0.27d	0.32c	0.28b
Subsoil-bedding	0.41c	0.72b	0.69ab	0.35bc	0.32c	0.38bc	0.54bc	0.62ab	0.50ab
Shred-bedding	0.40cd	0.64b	0.68ab	0.38bc	0.37c	0.45b	0.39c	0.46bc	0.60a
No tillage	0.77b	0.87b	0.94a	0.48b	0.49b	0.43b	0.60b	0.39c	0.37ab

*Values in columns followed the same letter do not differ significantly ($p<0.05$) as determined by the LSD test.

The most unstable soil was that under mouldboard ploughing, at all soil depths (Table 2). Aggregate stability generally remained constant with soil depth in all the cultivated soils. The loss of soil organic matter promoted by tillage in the mouldboard soil could be responsible for the fact that the lowest aggregate stability occurred in this soil. However, changes in aggregate stability following land use changes have been observed without changes in total soil organic matter content. These results may indicate that only some soil organic matter fractions are involved in soil structural stability or that stability is quicker to change than total organic carbon. For example, in the water soluble C fraction there are extracellular polysaccharides, from bacteria or fungi, and root mucilages that act as binding agents of soil aggregates (Roldán *et al*., 1996). No tillage significantly increased crop residue accumulation on the soil surface, which enriched this soil in labile organic matter. No tillage may promote fungal growth and the proliferation of fungal hyphae that contribute to macroaggregate formation (Roldán *et al*., 1994). Doran *et al*. (1980) indicated that populations of fungi were significantly higher in the surface (0-7.5 cm) of no tillage soils than in the surface of tilled soil. Likewise, the increase of soil aggregate stability in the no tillage treatments can be attributed to the increases observed in microbial activity of such soils. Reduced aggregation and increased turnover of aggregates in conventional tillage, compared to no tillage, are a direct function of immediate physical disturbance due to ploughing. Tillage continually exposes new soil to wet-dry cycles at the soil surface, thereby increasing the susceptibility of aggregates to further disruption. Furthermore, tillage changes soil conditions, such as temperature, moisture and aeration, and increases the decomposition rates of the litter. To a depth of 10 cm, the no tillage soil did not reach the percentages of stable aggregates of the soil under natural vegetation.

Table 2. Structural stability of the soil under natural vegetation and different management tillage systems (n=3).

	Aggregate stability (%)		
	0-5	5-10	10-20
	Soil depth (cm)		
Natural vegetation	43.5a*	35.5a	25.3a
Mouldboard	15.1c	13.8d	16.2b
Subsoil-bedding	25.4bc	26.1b	26.5a
Shred-bedding	24.4bc	15.5d	26.6a
No tillage	27.3b	22.1c	30.1a

*Values in columns followed the same letter do not differ significantly ($p<0.05$) as determined by the LSD test.

Conclusions

The enzyme activities and aggregate stability reflected early changes in the soil profile to a greater extent than did physical-chemical or chemical properties. The no tillage system, which promotes a great surface accumulation of crop residues, was the most effective for improving soil physical and biochemical quality, which may contribute greatly to long-term sustainability of agricultural ecosystems under subtropical conditions. Over the duration of this experiment, this conservation tillage system was still far from reaching the quality levels of the soil under natural vegetation.

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