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Soil enzyme activities suggest advantages of conservation tillage practices in sorghum cultivation under subtropical conditions

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Abstract

Soil enzyme activity can be used as an indicator of soil quality for assessing the sustainability of agricultural ecosystems. 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), and conventional tillage practices, such as mouldboard ploughing, on physical–chemical, biochemical and physical soil quality indicators in a degraded sorghum field under warm subtropical conditions, after a period of 3 years. An adjacent soil under native vegetation was used as a standard representing local high quality soil. Conservation tillage systems, in particular no tillage, increased crop residue accumulation on the soil surface. Soil electrical conductivity and pH were not affected by the tillage practices. In the 0 to 5 cm layer, organic matter content increased with decreasing tillage intensity and was 33% greater with no tillage compared with the average of the other tillage treatments. The no tilled soil had higher values of water soluble C, dehydrogenase, urease, protease, phosphatase and β-glucosidase activities and aggregate stability than tilled soils, but had lower values than the soil under native vegetation. The enzyme activity and aggregate stability showed higher sensitivity to soil management practices than did physical–chemical properties. The no tillage system was the most effective for improving soil physical and biochemical qualities.

Keywords: Aggregate stability; Crop residue; Microbial activity; No tillage; Soil enzyme activities

1. 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. There are about 650 thousand

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hectares under conservation tillage in Mexico, representing nearly 3.25% of the agricultural area under annual crops (Claveran-Alonso et al., 2001). Conservation tillage refers to several practices based on the use and management of crop residues for covering at least 30% of the soil surface, preventing or minimising problems with erosion and degradation. Some of those practices are no tillage and reduced/minimum tillage, with or without incorporation of crop residues. Application of conservation tillage systems presents several beneficial effects, such as reduction of erosion (Tiscareño et al., 1999), weed problems and chemical fertiliser use, and restoration of soil fertility (Salinas-García et al., 2002), which are fundamentally due to crop residue.

Numerous studies have been conducted on the effects of tillage on the total amount and distribution of soil organic matter. In general, agricultural disturbance of soil has led to soil organic matter losses, but primarily in soils of warm, wet tropical and subtropical regions (Bayer et al., 2001). The soil organic matter shortage results in declines of soil quality and crop productivity, since organic matter is sink and source of nutrients, enhances soil physical and chemical properties and determines biological activity (Gregorich et al., 1994). The influence of soil tillage systems on the total soil organic matter content is detectable experimentally only after a long period of time. Microbial activity-based indicators of soil quality may respond to disturbances on a shorter period of time than those based on physical or chemical properties. As a consequence, microbiological properties, such as soil enzyme activities have been suggested as potential indicators of soil quality because of their essential role in soil biology, ease of measurement and rapid response to changes in soil management (Kandeler et al., 1999). There are a number of studies on the effects of different tillage management systems on biological characteristics of semiarid Mediterranean and temperate soils (Kandeler et al., 1999; Riffaldi et al., 2002). However, no information is available on changes in soil enzyme activities due to 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), and conventional tillage practices, such as mouldboard ploughing, on physical–chemical, biochemical and physical soil quality indicators in a degraded sorghum field under warm subtropical conditions. An adjacent soil under native vegetation was used as a standard of local high quality soil.

2. Materials and methods

2.1. Site description

This research was conducted at the Río Bravo experimental site, in Northern Tamaulipas, Mexico (25°57′N, 98°01′W). The dominant soil type is Vertisol (FAO, 1988) developed from alluvial sediments with a clay texture (28% sand, 31% silt, and 41% clay), 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 23 °C 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.

2.2. 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 m×52.0 m. The examined tillage treatments 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.

2.3. Soil sampling

Soil samples were collected during the 2003 sorghum growing season, in late April. Soil samples from each plot were composed from five subsamples, that were taken with a probe (6.0-cm diameter core) and divided into segments of 0 to 5, 5 to 10, and 10 to 20 cm. As a standard of local high quality soil, a soil under native vegetation adjacent to the cultivated area was also collected. Field-moist soil samples were divided into two subsamples. One soil subsample was sieved at 2 mm and stored at 2 °C for biochemical analysis and another soil subsample was allowed to dry at room temperature for physical–chemical and physical analyses.

Surface crop residues were collected before primary tillage from two midrow to midrow 1 m^2 areas that were representative of each tillage treatment, dried (60 °C, 48 h), weighed and converted to Mg ha^{-1} (Steiner et al., 1994).

The percentage of surface cover was determined using a line-transect measurement, stretching a 10-m string with 100 marks (10 cm apart) across the field, at a 45° angle to the rows. Walking along the line, looking straight down, we counted the number of marks which coincided with surface residue. The number of "hits" indicates the percentage of cover (Steiner et al., 1994).

2.4. Physical–chemical analyses

Soil pH was determined in a 1:2 (w/v) soil–water extract. Electrical conductivity was measured on a saturated paste. Total organic nitrogen was determined by the Kjeldahl method, and the total organic C was determined by oxidation with potassium dichromate in a sulphuric medium and excess dichromate evaluated using Mohr's salt (Yeomans and Bremner, 1988). Water soluble C in soil aqueous extracts (1:5, w/v) was determined by wet oxidation with potassium dichromate followed by measurement of the absorbance at 590 nm (Sims and Haby, 1971). Available P, extracted with 0.5 M sodium bicarbonate, pH 8.5, was determined by colorimetry, according to Murphy and Riley (1962). Ammonium acetate extractable K was determined by cobaltinitrite method (Harris, 1940). NO_3–N was measured using the cadmium reduction method, following extraction with 2 M KCl (1:4 w/v) and shaking for 30 min (Technicon, 1977).

2.5. Biochemical analyses

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 solution of 0.4% INT (2-*p*-iodophenyl-3-*p*-nitrophenyl-5-phenyltetrazolium chloride) for 20 h, at 22 °C in darkness. The INTF formed (iodo-nitrotetrazolium formazan) was extracted with 10 mL of methanol by shaking vigorously for 1 min and filtration through a Whatman No. 5 filter paper. The INTF was measured spectrophotometrically at 490 nm.

Urease and *N*-α-benzoyl-L-argininamide (BAA) hydrolyzing protease activities were determined in 0.1 M phosphate buffer at pH 7; 1 M urea and 0.03 M BAA were used as substrates, respectively. Two milliliters of buffer and 0.5 mL of substrate were added to 0.5 g of sample, which was incubated at 30 °C (for urease) or 39 °C (for protease) for 90 min. Both activities were determined as the NH_4^+ released in the hydrolysis reaction (Nannipieri et al., 1980).

Acid phosphatase activity was determined using *p*-nitrophenyl phosphate disodium (PNPP, 0.115 M) as substrate. Two milliliters of 0.5 M sodium acetate buffer at pH 5.5 using acetic acid (Naseby and Lynch, 1997) and 0.5 mL of substrate were added to 0.5 g of soil and incubated at 37 °C for 90 min. The reaction 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 2287×*g* for 5 min. The *p*-nitrophenol (PNP) formed was determined by spectrophotometry at 398 nm (Tabatabai and Bremner, 1969). Controls were made in the same way, although the substrate was added before the $CaCl_2$ and NaOH.

β-glucosidase was determined using *p*-nitrophenyl-β-D-glucopyranoside (PNG, 0.05 M) as substrate. This assay is based on the release and detection of PNP. Two milliliters of 0.1 M maleate buffer at pH 6.5 and 0.5 mL of substrate was added to 0.5 g of sample and incubated at 37 °C for 90 min. The reaction was stopped with tris-hydroxymethyl aminomethane (THAM) according to Tabatabai (1982). The amount of PNP was determined in a spectrophotometer at 398 nm (Tabatabai and Bremner, 1969).

2.6. Physical analyses

The percentage of water stable aggregates was determined by the method described by Lax et al. (1994). A 4-g aliquot of sieved (0.20–4.00 mm) air-dried soil was placed on a 0.25 mm sieve (7.5-cm diameter) and wetted by spray. After 15 min the soil was subjected to an artificial rainfall of about 34 mm with an energy of 270 J m^{-2}. The remaining soil on the sieve was dried at 105 °C and weighed (P1). Then, the soil was soaked in distilled water and, after 2 h, passed through the same 0.25 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 single particles, was dried at 105 °C and weighed (P2). The percentage of stable aggregates relative to the total aggregates was calculated as (P1−P2)×100/(4−P2).

Bulk density was calculated from dry soil weights (105 °C, 48 h) and the volume of samples taken with a hammer-driven core sampler (55-mm diameter; Blake, 1965).

2.7. 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.

3. Results and discussion

3.1. Surface crop residue

Surface crop residue was significantly affected by tillage practices (Table 1). No tillage enabled the highest residue amount (on average about 42 times more residue than mouldboard plough) and cover (95%) on the soil surface, while soils under mouldboard ploughing showed the least amount and covering residue. Subsoil-bedding was significantly more effective for increasing surface crop residue amount than shred-bedding. Long-term tillage practices which do not incorporate crop residues and allow then to accumulate on soil surface have been shown to reduce erosion and improve the soil water retention (Tiscareño et al., 1999).

Table 1
Surface crop residue as affected by tillage systems

	Residue cover (%)	Residue amount (Mg ha^{-1})
Mouldboard	2d[1]	0.17d
Subsoil-bedding	27b	2.29b
Shred-bedding	15c	2.16c
No tillage	95a	7.13a

[1] Values in columns, followed by the same letter, are not significantly different ($p<0.05$), as determined by the LSD test.

3.2. Soil physical–chemical properties

Electrical conductivity and pH values were similar in the soil under natural vegetation and those under different tillage practices (Table 2). Roldán et al. (2003) also reported no changes in soil electrical conductivity after 6 years of conservation practices in a maize field.

In the 0 to 5 cm layer, organic matter content increased with decreasing tillage intensity so that it was 32% greater with no tillage, compared to the average of the other tillage treatments (Table 2). Mouldboard ploughing resulted in the lowest organic matter content throughout the 0–20 cm soil layer. Below the 0 to 5 cm layer, organic matter decreased under no tillage, but tended to remain constant in the other treatments. The tillage treatments (mouldboard, subsoil-bedding and shred-bedding), compared to no-tillage, incorporate residues into a larger volume of soil and therefore increase the rate of organic matter decomposition and C mineralisation (Salinas-García et al., 2002), by increasing the contact between soil microorganisms and crop residues (Henriksen and Breland, 2002). It is worth noting that in all layers no tillage soil had organic matter levels similar to the soil under natural vegetation.

Soil nitrate concentration was consistently lower in no-tilled soil than in tilled ones (Table 2). Reduced soil tillage often results in a lower $N–NO_3^-$ content in the rooting zone, compared with conventional tillage (Doran, 1980). Since soil under no tillage is not disturbed, the organic N mineralisation is significantly reduced and so is the concentration of inorganic N forms. On the other hand, the lower $N–NO_3^-$ concentration could be due to less aerobic conditions in the no-tilled soil, which result in higher losses of N

Table 2
Physical–chemical properties, organic matter and nutrients concentration of the soil under native vegetation and different 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
	pH (H_2O)			Electrical conductivity (mS cm^{-1})			Total organic carbon (%)		
Native vegetation	8.00a[1]A[2]	8.14aA	8.23aA	0.80aA	0.80aA	0.80aA	2.2aA	1.7aB	1.6aB
Mouldboard	8.07aA	8.03aA	8.17aA	1.37aA	0.90aB	0.93aAB	1.3bA	1.2cA	1.2cA
Subsoil-bedding	8.03aA	8.13aA	8.07aA	1.40aA	1.13aA	1.33aA	1.5bA	1.5abA	1.3bA
Shred-bedding	8.03aA	8.13aA	8.03aA	0.97aA	0.83aA	0.73aA	1.5bA	1.4bA	1.3bcA
No tillage	8.00aA	8.00aA	8.05aA	1.17aA	0.90aA	1.10aA	1.9aA	1.6aB	1.5aB
	$N–NO_3^-$ (μg g^{-1})			Available P (μg g^{-1})			Extractable K (μg g^{-1})		
Native vegetation	5bA	3bB	2dB	14aA	13aA	13aA	533aA	503aAB	433aB
Mouldboard	10aA	10aA	11aA	12aA	12aA	12aA	390aA	410aA	460aA
Subsoil-bedding	11aA	10aA	10abA	12aA	11aA	12aA	393aA	433aA	447aA
Shred-bedding	11aA	9aAB	8bB	12aA	12aA	13aA	433aA	533aA	487aA
No tillage	6bA	4bB	4cB	12aA	13aA	12aA	403aA	417aA	453aA

[1] Values in columns, followed by the same small letter, do not differ significantly (p<0.05) as determined by the LSD test.
[2] Values in rows, followed by the same capital letter, do not differ significantly (p<0.05) as determined by the LSD test.

through denitrification. Tilled soils have significantly higher numbers of aerobic microorganisms and nitrifiers than non-tilled soils (Doran, 1980). Nitrate did not vary with soil depth in the soils under mouldboard or subsoil-bedding tillage, which may indicate either major nitrate leaching in these treatments or major homogeneity of nitrate within soil tilled. Only the soil under no tillage showed low nitrate levels similar to those of soil under native vegetation, to a depth of 10 cm. Available P and extractable K were not affected by any of the treatments or by soil depth (Table 2). Despite the higher accumulation of organic matter at the surface layer of no-tilled soil, available P did not increase there. The low solubility of phosphorus could be due to the fact that the soil exhibits a slightly alkaline pH (El-Baruni and Olsen, 1979). There were no differences in nutrient content (PK) between soils under natural vegetation and those under cultivation.

Like for total organic matter, tillage intensity also decreased the water soluble C (Table 3), which in mouldboard tilled soil was on average about 20% lower than in soil under no tillage. The study of this labile organic C fraction is fully relevant, particularly for agricultural soils, since it determines the soil microbial activity (Janzen et al., 1992) and plays a key role in the formation and stabilization of soil aggregates (Metzger and Yaron, 1987). Considering that, we can suppose that all tilled soils, and even the no tilled one, had less microbial activity than the soil under native vegetation.

3.3. Soil biochemical properties

Dehydrogenase activity decreased significantly with agricultural use of soil, particularly with intensive (mouldboard) and even with reduced tillage (subsoil-bedding and shred-bedding), as shown in Table 3. The differences in dehydrogenase activity 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 (Nannipieri, 1994) and a valid biomarker to indicate changes in total microbial activity due to changes in soil management (Ceccanti et al., 1994). Dehydrogenase activity responded to the treatments in a similar to water soluble C, i.e. increasing with adoption of no tillage, in direct proportion to the accumulation of crop residues on the soil surface. 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.

Soil hydrolases can provide early indications about soil fertility status, since those enzymes are related to the mineralisation of important nutrient elements such as N, P and S. The use of a single enzyme in soil quality investigations has been criticised by several

Table 3
Water soluble C and enzyme activity of soil under native vegetation and different 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 $g^{-1}h^{-1}$)		
Native vegetation	187a[1]A[2]	199aA	173aA	100.2aA	68.9aB	40.9aC	1.93aA	2.18aA	2.15aA
Mouldboard	94cA	90cA	96bA	45.2cA	44.6cA	44.3aA	0.63cA	0.65cA	0.72bA
Subsoil-bedding	106bcAB	115bA	98bB	50.0cA	56.4abcA	51.1aA	0.62cA	0.77bcA	0.84bA
Shred-bedding	105bcA	115bA	105bA	48.5cA	51.7bcA	49.9aA	0.84bcA	0.70bcA	0.81bA
No tillage	113bA	118bA	104bB	66.1bA	57.7abAB	40.8aB	1.25abA	0.83bA	0.90bA
	Protease (μmol NH_3 $g^{-1}h^{-1}$)			Phosphatase (μmol PNP $g^{-1}h^{-1}$)			β-glucosidase (μmol PNP $g^{-1}h^{-1}$)		
Native vegetation	2.05aA	2.34aA	1.25aA	1.18aA	1.03aB	0.74aC	1.47aA	0.85aB	0.31abC
Mouldboard	0.23dB	0.31cAB	0.43bA	0.29cA	0.30cA	0.29cA	0.27dA	0.32cA	0.28bA
Subsoil-bedding	0.41cB	0.72bA	0.69abA	0.35bcA	0.32cA	0.38bcA	0.54bcAB	0.62abA	0.50abB
Shred-bedding	0.40cdB	0.64bA	0.68abA	0.38bcA	0.37cA	0.45bA	0.39cB	0.46bcB	0.60aA
No tillage	0.77bB	0.87bAB	0.94aA	0.48bA	0.49bA	0.43bA	0.60bA	0.39cB	0.37abB

[1] Values in columns, followed by the same small letter, do not differ significantly (p<0.05) as determined by the LSD test.
[2] Values in rows, followed by the same capital letter, do not differ significantly (p<0.05) as determined by the LSD test.

authors, mainly because one enzyme catalyses specific reactions and is 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 3). 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). With conventional tillage, organic matter is more uniformly distributed into the soil than with reduced tillage, where crop residues are concentrated on the soil surface (Salinas-García et al., 2002). As a consequence, the microbial activity is also uniformly distributed along the plough layer in the tilled soils. However, in no-tilled soils there was no clear relationship between microbial activity and soil depth. Only the activity of protease, an enzyme involved in N cycling, increased significantly with depth in all the treatments (Table 3). The lack of a depth effect on acid phosphatase activity, which is involved in P cycling, is most likely because this enzyme is predominantly secreted by plant roots and associated mycorrhiza and other fungi (Tarafdar and Marschner, 1994). Similar results with respect to acid phosphatase activity were reported by Naseby and Lynch (1997). No tillage produced lower hydrolase activities, to a depth of 10 cm, compared to the soil under natural vegetation.

3.4. Soil physical properties

The most unstable soil was that under mouldboard ploughing (Table 4). Aggregate stability of all the cultivated soils generally remained constant with depth. The loss of organic matter could be the reason of the lowest aggregate stability in mouldboard-ploughed soil. However, changes in aggregate stabil-

Table 4
Physical properties of the soil under native vegetation and different tillage systems (n=3)

	Soil depth (cm)					
	0–5	5–10	10–20	0–5	5–10	10–20
	Aggregate stability (%)			Bulk density (g cm^{-3})		
Native vegetation	43.5a[1]A[2]	35.5aB	25.3aC	1.21bB	1.27bB	1.48aA
Mouldboard	15.1cA	13.8dA	16.2bA	1.15cC	1.22bB	1.54aA
Subsoil-bedding	25.4bcA	26.1bA	26.5aA	1.23bC	1.34aB	1.50aA
Shred-bedding	24.4bcAB	15.5dB	26.6aA	1.26abC	1.39aB	1.54aA
No tillage	27.3bA	22.1cA	30.1aA	1.29aC	1.38aB	1.53aA

[1] Values in columns, followed by the same small letter, do not differ significantly (p<0.05) as determined by the LSD test.
[2] Values in rows, followed by the capital same letter, do not differ significantly (p<0.05) as determined by the LSD test.

ity, following land use conversion have been observed without alterations in total soil organic matter content (Puget et al., 1999). These results may indicate that only some soil organic matter fractions are involved in soil structural stability or that stability is easier 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 (Beare et al., 1994; Lu et al., 1998). No tillage may promote fungal growth and the proliferation of fungal hyphae that contribute to macroaggregate formation (Roldán et al., 1994). Doran (1980) indicated that biomass of fungi was 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 (Beare et al., 1994), 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.

Mouldboard ploughing reduced the bulk density down to a depth of 10 cm (Table 4). The bulk density increased with soil depth and in the deepest (10 to 20 cm) layer it was practically the same among the tillage treatments. The high clay content in this type of soil and the accumulated effects of machinery traffic during the no tillage operations lead to an important compaction, which can be reduced effectively by tillage. Likewise, the slow incorporation of organic matter from crop residues in the no tillage soil, due to the rapid decomposition rate of such residues under subtropical conditions, impeded the decrease of soil bulk density during the short duration of the experiment. Thus, no-tilled soil reached values of bulk density higher than that of soil under native vegetation. However, structural stability of no tilled soil remains important (22% to 30%) and the relative compaction seems not to be an obstacle to deep rooting of the crops or seedling emergence.

4. Conclusion

The enzyme activity and aggregate stability showed higher sensitivity to soil management practices than did physical–chemical properties. The no tillage system, which allows a great surface accumulation of crop residues, was the most effective for improving soil physical and biochemical qualities and therefore may contribute to a long-term sustainability of agricultural ecosystems under subtropical conditions. However, 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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