DÍAZ-RAVIÑA, M.¹, BUENO, J.², GONZÁLEZ-PRIETO, S.J.¹, CARBALLAS T. (2005)¹. Cultivation effects on biochemical properties, C storage and ¹⁵N natural abundance in the 0-5 cm layer of an acidic soil from temperate humid zone. *Soil Tillage Research* **84**, 216-221.

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Abstract.- Changes in some soil chemical, including ¹⁵N values, and biochemical properties (microbial C, FDA hydrolysis, glucosidase and urease activities) due to two tillage systems, conventional tillage (CT) and no tillage (NT), were evaluated in an acid soil from temperate humid zone (NW of Spain) and compared with values obtained for a reference forest soil. The results showed that in the surface layer (0-5 cm depth) tillage tended to increase soil pH and to decrease organic matter levels and microbial biomass and activity values. The data also indicated that 8 years of NT, compared to CT, resulted in greater organic matter content and increased microbial biomass and activity, the changes being more pronounced for the microbial properties. Adoption of NT resulted in an increase of soil C storage of 1.24 Mg C ha⁻¹ yr⁻¹ with regard to CT. The suitability of ¹⁵N as a potential tracer of land use in this acid soil was also confirmed.

Key words: Microbial biomass; Soil enzymes; ¹⁵N abundance; C sequestration; Tillage

Introduction

Soil organic matter (SOM) is an important soil quality attribute because of its influence on physical, chemical and biological properties and processes that, in turn, contribute to improve soil productivity (Gregorich et al., 1994). Recent concern for world-wide climate change has also increased the interest in SOM and its role in the global C budget through sequestration of atmospheric C (Lal, 2001). Assessment of SOM is therefore a valuable step to identify the overall quality of a soil and the sustainability of land management. Studies of soil ecosystems under long-term management involving different tillage practices as well as undisturbed native soils have demonstrated that tillage may cause a substantial decrease of SOM content and labile pools of nutrients (Elliot, 1986; Karlen et al., 1994; Wander and Bollero, 1999). On the contrary, the change of tillage methods to reduced- or no-tillage practices is recommended to sequester organic C and hence to reduce the net emission of greenhouse gases (Lal, 2002). Short- and medium-term changes in SOM following a change in soil management or land use are less well understood because they are difficult to measure by conventional methods.

In the temperate humid zone of NW Spain conversion of natural forest to arable lands and pastures is the most widespread change in land use in the past hundred years. Therefore, large areas of forest are being cultivated with crop sequences under conventional tillage. Most of these crop rotations are considered to be strongly degrading systems because of the negative effect of intensive tillage on SOM, soil structure, etc., and the small quantity of plant residues on the soil after harvesting. However, information on the impact of tillage systems on properties of these acidic soils is scarce and is mainly limited to physical properties (Benito and Díaz-Fierros, 1992). At present, conservation tillage is being adopted in this area as an alternative to restore soil structure and fertility. The aim of this work was to evaluate whether changes in soil chemical, including ¹⁵N values, and in biochemical properties due to two tillage systems (conventional tillage and no-tillage) could be detected after 8 years of maize-ryegrass rotation. Soil properties were also analysed using the same soil without disturbance as reference.

Material and methods

The experimental field was located in the Gayoso-Castro farm (43°06'N, 7°27'W, 420 m a.s.l.) at Castro de Ribeiras de Lea (Galicia, NW Spain). The soil is a Gleyic Cambisol with sandy loam topsoil. Tillage treatments were arranged in a complete randomised block design with four replications and 2 m separation established around each plot (20 m x 5 m). Since 1994, the same annual ryegrass-maize rotation has been

cultivated under two tillage systems, conventional tillage (CT) and no tillage (NT). Silage maize is being sown in rows 0.7 m apart in late May and harvested in September. Before sowing, plants established in the NT treatments were destroyed with glyphosate application at a dose of 5 L ha⁻¹ whereas in the CT treatments they were buried by ploughing at 25-30 cm. Further agrochemical applications were identical for both treatments (CT, NT): one mixture of herbicides (33% acetachlor and 16.5% atrazine), insecticide (clorpiriphos) and 12-12-24 NPK at rates of 4 L ha⁻¹, 0.33 L ha⁻¹ and 700 kg ha⁻¹, respectively. Measurements of chemical and biochemical properties were carried out on all soil samples collected before sowing (0 time) and at different time intervals during the maize cropping (2, 4 and 12 weeks after sowing). In each sampling plot, samples were taken at 0-5 cm depth from 16 points uniformly distributed in the central rows between the maize plants and mixed to obtain a composite sample per plot. Additionally, a control soil was sampled at random in an adjacent (50 m apart) forest soil without human disturbance during the last 60 y. After sieving at 4 mm, the homogenized soil samples were stored at 4°C prior to further analyses of biochemical properties.

Microbial biomass C was determined using the method described by Vance et al. (1987). Enzyme activities of several hydrolases were measured as indicators of soil metabolic activity. Fluorescein diacetate (FDA) hydrolysis, β -glucosidase and urease activities were assessed as reported by Schnürer and Rosswall (1982), Eivazi and Tabatabai (1988) and Kandeler and Gerber (1988), respectively. Total N content and $\delta^{15}\text{N}$ values of soils were measured on finely ground samples (< 100 μm) with an elemental analyser (EA) coupled on-line with an isotopic ratio mass spectrometer (Finnigan Mat, delta C, Bremen, Germany). Total inorganic N was measured in 2 M KCl extracts and extractable C in 0.05 M K₂SO₄ (Basanta et al., 2002).

All results were obtained by triplicate determinations and were expressed on the basis of oven-dry (105 °C) weight of soil. Data obtained at different field plots (4 field replicates per treatment) were statistically analysed by standard analysis of variance (ANOVA 1) and, in cases of significant F values, Tukey's minimum significant difference test was used to separate the means. For cultivated soils, the percentage of data variation attributable to soil management and sampling time was calculated using two-way analysis of variance.

Results and discussion

Main chemical and physical-chemical properties of the 0-5 cm soil samples analysed at different sampling times are summarized in Table 1. Inorganic N data did not show a clear pattern and water holding capacity values were often higher in soils with higher organic matter content. Compared with the reference forest soil (NC), in the upper 5 cm of both cultivated soils (CT and NT) the values for total C, total N, C/N and extractable C were significantly lower (decrease of 30-65%, 16-51%, 21-32% and 44-58%, respectively) and pH was significantly higher (1.4-2.1 units). Compared with the CT treatment, the pH values were similar or slightly lower in the NT treatment whereas total C, total N and hence moisture content were significantly much higher at all sampling times.

Table 1. Main characteristics of the 0-5 cm soil layer from a cultivated soil under conventional (CT) or no-tillage (NT), and an adjacent non cultivated (NC) soil. (Mean values of four experimental plots $\pm$ SD).

Time after sowing (weeks)	Treatment	WHC (g kg ⁻¹)	pH (H ₂ O)	Total C (g kg ⁻¹)	Total N (g kg ⁻¹)	$\delta^{15}\text{N}$	Inorganic N (mg kg ⁻¹)	Extractable C (mg kg ⁻¹)
0	NC	563 $\pm$ 2	4.4 $\pm$ 0.1	111.1 $\pm$ 7.6	5.3 $\pm$ 0.6	0.76 $\pm$ 0.05	12.6 $\pm$ 0.1	15.6 $\pm$ 0.5
	CT	305 $\pm$ 2	5.8 $\pm$ 0.1	39.4 $\pm$ 4.4	2.7 $\pm$ 0.2	4.93 $\pm$ 0.18	1.4 $\pm$ 0.3	7.6 $\pm$ 0.2
	NT	410 $\pm$ 1	5.7 $\pm$ 0.1	56.3 $\pm$ 4.5	3.4 $\pm$ 0.4	4.16 $\pm$ 0.20	3.1 $\pm$ 2.1	7.8 $\pm$ 0.2
2	NC	494 $\pm$ 2	3.8 $\pm$ 0.1	97.3 $\pm$ 4.5	5.2 $\pm$ 0.5	1.10 $\pm$ 0.32	9.8 $\pm$ 1.0	15.4 $\pm$ 2.3
	CT	302 $\pm$ 2	5.8 $\pm$ 0.1	38.5 $\pm$ 2.1	2.9 $\pm$ 0.2	4.74 $\pm$ 0.12	8.6 $\pm$ 1.3	8.2 $\pm$ 0.5
	NT	378 $\pm$ 4	5.6 $\pm$ 0.2	55.2 $\pm$ 4.5	3.7 $\pm$ 0.4	4.11 $\pm$ 0.20	8.7 $\pm$ 1.8	8.4 $\pm$ 0.7
4	NC	504 $\pm$ 2	3.9 $\pm$ 0.1	96.7 $\pm$ 1.0	5.1 $\pm$ 0.5	0.87 $\pm$ 0.27	8.3 $\pm$ 0.9	14.2 $\pm$ 0.3
	CT	317 $\pm$ 2	6.2 $\pm$ 0.1	40.3 $\pm$ 1.0	3.0 $\pm$ 0.1	4.61 $\pm$ 0.10	15.2 $\pm$ 2.3	7.0 $\pm$ 0.4
	NT	419 $\pm$ 3	5.8 $\pm$ 0.4	54.8 $\pm$ 2.6	3.8 $\pm$ 0.5	4.35 $\pm$ 0.24	11.5 $\pm$ 2.5	6.3 $\pm$ 0.8
12	NC	519 $\pm$ 2	4.1 $\pm$ 0.1	110.5 $\pm$ 3.5	5.4 $\pm$ 0.6	0.55 $\pm$ 0.21	n. d.	19.1 $\pm$ 1.1
	CT	331 $\pm$ 1	5.6 $\pm$ 0.1	43.2 $\pm$ 1.2	2.8 $\pm$ 0.2	4.92 $\pm$ 0.10	n. d.	10.4 $\pm$ 0.8
	NT	428 $\pm$ 3	5.3 $\pm$ 0.1	55.9 $\pm$ 2.7	3.7 $\pm$ 0.3	4.92 $\pm$ 0.14	n. d.	11.1 $\pm$ 0.6

WHC, water holding capacity; n.d., not determined

The effect of soil management on C stock was determined by multiplying the mean values obtained at different sampling times with concurrently measured soil bulk densities (average values were 1.00, 1.13 and 1.17 g cm⁻³ for NC, CT and NT treatments, respectively). The C content of the 0-5 cm layer was 51.9 Mg C ha⁻¹ in NC soil, 22.8 Mg C ha⁻¹ in CT treatment and 32.7 Mg C ha⁻¹ in NT treatment. Therefore, during the last 60 y, the C content in the 0-5 cm layer of NC and CT soils diverged by 29.1 Mg C ha⁻¹. Although a net increase of C content in NC can not be discarded, this difference seems largely due to a wide decrease in the C content of CT. This diminution can be notably mitigated by the adoption of conservation tillage practices since after 8 years of NT management C stock in the 0-5 cm soil layer had increased about 9.9 Mg C ha⁻¹ relative to that in the CT treatment (gain of 43%), which means 1.24 Mg C ha⁻¹ year⁻¹. This is in accordance with other studies showing the effectiveness, particularly in humid regions, of reduced- and no-tillage systems for C sequestration and hence for mitigating climate change (Dick et al., 1998; Follett, 2001; Lal, 2002). However, since the depth distribution of C varied with tillage system (Angers et al., 1997; Franzluebbers, 2002), SOM content should be examined through the whole soil profile to confirm this hypothesis.

Like the total C content, soil total N levels in the upper 5 cm of CT plots were consistently lower than those in the NC and NT plots (Table 1; Fig. 1). Conversely, the $\delta^{15}\text{N}$ natural abundance showed the opposite trend, with the lowest values in NC soils and the highest in CT soils, NT soils having slightly, but significantly, lower values than CT soils. These results agreed with the strong negative correlation normally found between the soil N and its $\delta^{15}\text{N}$ isotopic signature (Kerley and Jarvis, 1997; González-Prieto and Villar, 2003), suggesting that the difference in soil N content among NC, CT and NT plots are related with differences in N losses, which discriminate against the heavy isotope ¹⁵N (Högber et al., 1995). Therefore, total soil N and $\delta^{15}\text{N}$ data in the 0-5 cm soil layer suggested that the nearly 'closed' N cycling in the NC plots is widely disrupted in CT plots and that NT management contributes significantly to reduce the N losses due to cultivation. Consequently, NT management mitigates the negative environmental impacts of agriculture by reducing N losses through NO₃ leaching and/or NO_x emissions that contribute to eutrophication, acid rain, greenhouse effect and stratospheric O₃ depletion (IPCC, 1992).

Fig. 1. Differences between soil total N and $\delta^{15}\text{N}$ isotopic signatures (mean $\pm$ S.D.) in the 0-5 cm layer of a soil under conventional tillage (CT) or no-tillage (NT), and in an adjacent uncultivated soil (NC), at different sampling times (0, 2, 4 and 12 weeks after sowing).

The biochemical properties values obtained for the 0-5 cm soil layer of all soil treatments are shown in Fig. 2. The microbial C values ranged from 12 to 79 g C g⁻¹ d.w. and represented between 0.30 and 0.95% of the total C. The values in the NT and CT treatments were 31-48% of those in the corresponding control

(range of 48 to 79 $\mu\text{g C g}^{-1}$ d.w., 12 to 31 $\mu\text{g C g}^{-1}$ d.w. and 21 to 53 $\mu\text{g C g}^{-1}$, for NC, CT and NT management, respectively). For cultivated soils, 53% of variance in microbial C values was explained by tillage system whereas sampling time accounted for a further 26% of variance. The range of values for enzyme activities were 25-93 $\text{mg fluorescein g}^{-1}$ dry soil h^{-1} , 130-491 $\text{mg p-nitrophenol g}^{-1}$ dry soil h^{-1} and 22-88 $\text{mg NH}_4^+ \text{g}^{-1}$ dry soil h^{-1} for FDA hydrolysis, glucosidase and urease activities, respectively. As for microbial C, FDA hydrolysis values were higher in the NC soil, intermediate in soil under NT and lower under CT at all sampling times. For urease and glucosidase activities, values in NC soil were similar or even lower than those in NT soil whereas CT soil showed much lower values. Soil tillage system explained 66-85% of variation in these enzyme activities whereas sampling time accounted for only 8-14% of the variation, the interaction between these two factors being no significant.

Fig 2. Microbial biomass and enzyme activities (mean $\pm$ S.D.) in the 0-5 cm layer of a soil under conventional tillage (CT) or no-tillage (NT), and in an adjacent uncultivated soil (NC) at different sampling times (0, 2, 4 and 12 weeks after sowing).

The low values found for biochemical properties in the upper 5 cm of cultivated soils, particularly those under CT, as compared to reference forest soil are consistent with studies of several authors showing a negative influence of tillage, which increases with cultivation intensity (Doran, 1987; Granatstein et al., 1987; Dick, 1992; Kandeler et al., 1999; Wander and Bollero, 1999). The variations found over time are consistent with previous studies on soils located in the same area showing seasonal changes (Díaz-Raviña et al., 1993, 1995) and can be explained by fluctuations of different factors such as climatic conditions (temperature, moisture) and availability of substrate derived from roots or from material incorporated to the soil. In addition, in agricultural systems the effects of soil management (ploughing, agrochemical addition, residue incorporation) can also contributed to this temporal variation. The comparison of two

different tillage systems indicated significant differences ($p < 0.005$) at all sampling dates for all biochemical properties, the values in the CT treatments being 48-69% of those in the NT treatments. Differences in soil temperature and organic matter content and hence in substrate supply and associated soil physical properties may be major factors responsible for differences between tillage systems in biochemical properties (Doran, 1987; Biederbeck et al., 1994). This is directly related to surface accumulation of crop residues promoted by conservation tillage management, the total C and total N values in the 0-5 cm soil layer of CT treatments being 70-80% of those in the NT systems (Table 1). The positive correlations of SOM content with biomass C ($r = 0.87$, $P < 0.001$, $n = 12$), FDA hydrolysis ($r = 0.86$, $P < 0.001$) and enzyme activities ($r = 0.58$ and 0.55 for urease and glucosidase, respectively, $P < 0.05$) seem to support this assumption. The data also showed that the soil biochemical properties (e.g. microbial biomass and enzyme activities) were more sensitive than total organic C and N for assessing the impact of different tillage practices on soil quality, which is in accordance with studies of several authors (Carter, 1986; Saffina et al., 1989; Biederbeck et al., 1994; Bergstrom et al., 1998). The use of the biochemical properties analyzed is therefore recommended to detect changes in SOM trend due to land use and soil management over short- and medium-time scales.

Conclusion

Our data of chemical, biochemical properties and $\delta^{15}\text{N}$ natural abundance in the surface layer (0-5 cm) of an acidic soil located at the temperate humid zone (NW Spain) were coincident and clearly showed a decrease of soil quality upon conversion from natural to agricultural soils and a further improvement of soil quality by adoption of no tillage systems over a 8 years period as compared to conventional tillage. The results also seem to indicate that soil management has important implications for C sequestration.

Acknowledgements.—The authors thank Nuria Pérez Balsa and Angela Martín for technical assistance. This study was supported by Secretaría Xeral de Investigación e Desenvolvemento da Xunta de Galicia, Spain. The isotopic ratio mass spectrometer was partly financed by the European Regional Development Funds (EU).

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