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Particulate organic matter, carbohydrate, humic acid contents in soil macro- and microaggregates as affected by cultivation

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Abstract

Cultivation is known to strongly affect not only soil structure but also organic substances responsible for aggregation. This research was conducted to study the effect of cultivation on the distribution of soil macro- and microaggregates as related to changes in soil organic matter content in a Typic Haplustoll, located in central Cordoba, the principal peanut (*Arachis hypogaea* L.) growing area of Argentina. Samples were taken from A or Ap horizons at (1) an undisturbed and (2) a cultivated site for determination of the aggregate fractions by the wet sieving method. The mineral associated organic carbon (MOC) and particulate organic carbon (POC), in soils and their aggregates, were separated by dispersion and sieving procedures. Carbohydrate content in soil and in aggregate fractions was determined by dilute acid (CHda) and hot water (CHhw) extraction, whereas humic (HA) and fulvic acid (FA) were extracted by the NaOH method. The results indicated that the total macroaggregates (>250 μm) content was 1.7 times lower in the cultivated than in the undisturbed soil. The large macroaggregates (2800–2000 μm) were the most affected, decreasing a 92% due to cultivation compared with undisturbed. In contrast, the microaggregate (250–53 μm) content was twice that high in the cultivated than in the undisturbed soil. The concentrations of OC, POC, CHda, CHhw were all reduced substantially by cultivation, with the microaggregates showing an almost complete loss of its POC content. The destruction of these transient organic cementing agents was assumed to have contributed to the collapse of the macroaggregates. This has resulted in exposure of POC, making it more available to rapid oxidation and microbial attack. There were indications suggesting POC and CHhw contents to be valuable as indicators of soil structure degradation due to exhaustive cultivation practices. Although it is well know that humic substances are chemically and structurally much more stable than nonhumic substances but our results showed a surprising decrease humic substances under continuous cultivation.

Keywords: Aggregates; Humic substances; Organic matter; Cultivation; Soil degradation

1. Introduction

Organic matter (OM) is one of the most important constituents of soils due to its capacity in affecting plant growth indirectly and directly. Indirectly, OM improves the physical conditions of soils by enhancing aggregation, aeration and water retention, thereby creating a suitable environment for root growth (Senesi and Loffredo, 1999).

The relationship between OM and soil aggregation or structure formation was described by Tisdall and Oades (1982) in a conceptual model, affected by three types of aggregation agents. In soils, where the OM is the main

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binding agent, aggregates of different sizes can be formed. Primary particles and clay microstructure are bound together with bacterial and fungal debris into extremely stable microaggregates which may be bound together with fungal and plant debris giving a larger microaggregates. The humic matter, considered as a persistent cementing agent, is involved in stabilizing microaggregates. These microaggregates are bound into macroaggregates, due to the effect of transient binding agents (polysaccharides derived from plants and microorganisms) and temporary binding agents (fungal hyphae, fine roots, bacterial cells) (Tisdall and Oades, 1982; Oades, 1993). Particulate organic matter (POM) improves the soil aggregation since it can form an organic core surrounded by clay, silt particles, and aggregates (Jastrow and Miller, 1997).

The macroaggregates are less stable than microaggregates to wetting or mechanical actions such as plowing, and their destruction by tillage may result in exposure of the inner core of organic substances (Golchin et al., 1997; Six et al., 2000), facilitating rapid oxidation and attack by microorganisms of these important binding agents (Elliott, 1986; Angers and Chenu, 1997).

Because of synthesis and mineralization occurring at the same time, the concentration of humic substances is generally at equilibrium and remain constant at the prevailing ecoclimatic condition. However, when the precursors of humic matter disappear, the changes occurring in its composition and chemistry can modify its resistance to decomposition (Stevenson, 1982). Due to lower condensation, new complex polymeric humic substances fail to develop, resulting in formation of fewer and considerably less resistant humic compounds, serving as binding agents, with the consequent collapse or destruction of the aggregates. Therefore, this investigation was conducted with the objectives to study and evaluate the influence of tillage on the distribution of aggregates and changes of the binding agents in soils.

2. Materials and methods

2.1. Soil

Soil samples were collected in spring of 1999 from a Typic Haplustol (coarse loamy, mixed thermic), located in central Córdoba at 32°36′S and 63°59′W, the principal peanut (*Arachis hypogaea* L.) growing area of Argentina.

The places for sampling of the undisturbed soil were randomly selected in three close areas in with a natural forest vegetation. The cultivated soil samples were taken from areas near to the undisturbed ones.

The cultivated soil had been tilled for 50 years using the disc plow for growing mainly peanut (*Arachis hypogaea* L.), soybean (*Glicine max* (L.) Merr.) and corn (*Zea mays* L.). In the last 2 years conventional tillage was replaced by no-till method for growing soybean. The soils at both areas have been developed from the same parent materials under the influence of a similar semi-humid climate in a flat landscape. Therefore, the differences in soil properties studied were assumed to be primarily attributed to soil use or cultivation. The samples were collected from the surface layer undisturbed (A: 0–10 cm) and cultivated (A_p: 0–17 cm). The first centimeter of soil was removed to eliminate the litter, and a 10 kg sample was taken. They were gently broken down by hand in the laboratory and sieved to pass a 2.8 mm sieve while still moist and air-dried.

2.2. Aggregate analysis

The sieved soils from each plot were used for the determination of four aggregate size fractions by the wet sieving method, employing a series of three sieves. Ten grams of air-dried soil were placed on top of the three sieves, stacked on top of one another, and gently suspended in water (at room temperature) for 5 min prior to sieving without prewetting. Aggregate disruption was accomplished by moving the sieves 3 cm vertically 50 times for a period of 2 min, being careful to break the water surface with each stroke. The method used for aggregate size separation was adapted from Cambardella and Elliott (1994). The size fractions obtained were (1) large macroaggregates of 2800–2000 μm in size, (2) small macroaggregates of 2000–250 μm, (3) microaggregates of 250–53 μm, and (4) the <53 μm fraction (silt-plus clay-size particles) that passed the bottom sieve and was collected by centrifugation.

2.3. Extraction of MOC, POC, CHda, and CHhw

The mineral-associated organic carbon (MOC) and particulate organic carbon (POC) contained in the whole soil and in each aggregate fraction was separated by dispersing the aggregate and whole soil with sodium hexametaphosphate and passing the dispersed samples through a 53-μm sieve (Cambardella and Elliott, 1992). The material remaining on the sieve consisted of sand and POC, whereas the soil slurry, passing the sieve, contained the MOC. The suspension in the slurry was centrifuged at 1000 rpm for 15 min, and the precipitate

was dried at 50 °C for analysis of OC. The difference between the MOC content values and the OC content obtained from soil or aggregate sample without sieving was considered equal to the OC retained by the sieve or the POC. Since the sand was separated from the mineral-associated fraction during the sieving, it was necessary to correct the OC values of this fraction for sand prior to calculating this difference. According to Elliott et al. (1991), correction for sand content is very important when comparing OC or carbohydrate contents between fractions.

Extraction of carbohydrates was performed employing two different methods. In one of the methods, a dilute acid hydrolysis (CHda) was used by which 1 g of whole soil or the aggregate fractions were treated with 10 ml 0.5 M H_2SO_4, heated at 80 °C for 24 h. In the other method, a hot water extraction (CHhw) was carried out with 1 g whole soil or the aggregate fractions, each suspended in 10 ml of distilled water. The mixture was heated at 80 °C for 24 h, and hydrolysis was attained by adding H_2SO_4 to obtain a 0.5 M concentration as in the dilute acid hydrolysis procedure. After hydrolysis by each method, each suspension was centrifuged at 10,000 rpm for 15 min (Angers and Mehuys, 1989; Puget et al., 1999). Carbohydrate contents of the extract were determined by spectrometry using the antrona spectrometric method with glucose as the standard (Brink et al., 1960).

2.4. Extraction of humic and fulvic acids

Humic substances (HS) were extracted from the whole soil and aggregate fractions by the 0.1 N NaOH method, using a soil/NaOH ratio of 1:10 (Tan, 1996; Lobartini et al., 1997). Humic acid (HA) was separated from fulvic acid (FA) by acidification of the HS solution to pH 1.5 with 6 N HCl. The precipitated HA was collected by centrifugation, and purified by washing three times with a dilute solution of HF + HCl, after which it was redissolved by adding 0.5 N NaOH to a pH = 8.0. The HA solution was then passed through columns of anionic and H-saturated cationic exchange resins, freeze dried, and its content determined gravimetrically. The FA was concentrated by the XAD-8 resin method, eluted with 0.1 N NaOH and passed through a column of H-saturated cationic exchange resins, after which it was freeze dried for determination of its concentration.

2.5. Organic carbon analysis

The organic carbon in the original soil samples and in the different aggregate fractions was determined by dry combustion with a LECO-carbon-analizer CR12 (LECO Corporation, St. Joseph, MI, USA). Since it was deemed necessary to correct the results of the OC content in the aggregates for sand content as suggested by Elliott et al. (1991), the sand content in each aggregate fraction and the whole soil was determined using a pipet method (Gee and Bauder, 1986).

2.6. Statistical analysis

Differences of results as affected by tillage treatments were tested by analysis of variance using Fisher's LSD_{05} mean separation test at $p < 0.05$ (Steel and Torrie, 1980).

3. Results and discussion

3.1. Distribution of aggregate fractions

The distribution of the aggregate fractions, obtained by wet sieving, showed a drastic decrease of large macroagregates and a simultaneous increase of small soil aggregates due to cultivation (Table 1). The undisturbed soils were to be composed mostly of small macroaggregates (2000–250 μm) and the microaggregates (250–53 μm), mixed with relatively smaller amounts the large macroaggregates (2800–2000 μm). In contrast, the cultivated soil was composed dominantly of microaggregates and small macroaggregates, whereas the large macroaggregates were detected only in very small amounts. Such a shift in composition was attributed to the effect of cultivation breaking down the large macroaggregates into smaller size fractions, which is in accordance whit the aggregate hierarchy concept of Tisdall and Oades (1982). The large macroaggregates decreased drastically from 151.73 g kg^{-1} in the undisturbed soil to 12.13 g kg^{-1} in the cultivated soil. This considerable decrease means an almost total destruction (92%) of the large macroaggregates by cultivation. On the other hand, the microaggregates in the undisturbed

Table 1
Soil aggregate distribution and percentage of sand in undisturbed and cultivated sites

Fraction (μm)	Undisturbed		Cultivated	
	(g kg^{-1} soil)	(% sand)	(g kg^{-1} soil)	(% sand)
2800–2000	151.73*	24.98	12.13	26.85
2000–250	515.53*	43.98	378.11	46.09
250–53	296.26*	56.09	550.51	52.55
<53	30.34*	–	43.40	–
Whole soil		35.91		38.52

Values followed by * are significantly different within aggregate size between undisturbed and cultivated sites ($p < 0.05$), ns = not significant. <53 μm fraction content sand trace.

soil of 296.26 g kg^{-1} appeared to have increased considerably to 550.51 g kg^{-1} in the cultivated soil, which showed a whopping 50% increase due to cultivation.

3.2. Organic C (OC) and carbohydrate (CH) content

The OC content in whole soil decreased from 25.6 g kg^{-1} in undisturbed soil to 11.7 g kg^{-1} in the cultivated soil, which means a 46% decrease as a result of cultivation (Table 2). The OC concentration in the different aggregate fractions was also decreased substantially by cultivation except in the large macroaggregates fraction. The data indicated that the OC concentration in the large macroaggregates (2800–2000 μm) was lower than those in the small macroaggregates and the microaggregates fractions in the undisturbed soil. As shown in Table 2, cultivation has decreased these contents to more than 50% in the small macroaggregates and microaggregates.

The dilute acid extractable carbohydrate (CHda) concentration in whole soil decreased 47% because of cultivation (Table 2). Cultivation has also decreased 27% of the CHda contents in the 2800–2000 μm fraction and approximately 50% in the smaller aggregate fractions.

The concentrations of hot water soluble carbohydrate (CHhw) were apparently similarly affected by cultivation, except in the large macroaggregates. Because of their low values, the decrease due to cultivation seemed to be less obvious than that noted for CHda content. The lower contents in CHhw were due to the fact that the dilute acid procedure extracted soluble carbohydrates plus also carbohydrates from hemicelulose, whereas hot water extraction failed to produce the hydrolysis of the hemicellulose. The CHhw was mostly polysaccharides from plant exudates or microbial of origin (Angers and Mehuys, 1989; Haynes and Francis, 1993). In summary, it can be stated that the concentrations of OC, CHda and CHhw were all affected by cultivation in the whole soil and in the different aggregate fractions. When these transient and temporary cementing agents, responsible for binding together the microaggregates into macroaggregates, were destroyed by tillage, the large aggregate fraction was apparently broken down with the consequent increase in concentration of the 2000–250 μm fraction, and the 250–53 μm fraction in particular, as discussed in the previous section above.

Table 2
Organic carbon (OC), hot water soluble (CHhw) and dilute acid extractable (CHda) carbohydrate contents in the soil and aggregate fractions from undisturbed and cultivated sites

Fraction (μm)	Undisturbed			Cultivated		
	OC	CHda	CHhw	OC	Chda	CHhw
	(g kg^{-1} fraction)			(g kg^{-1} fraction)		
2800–2000	33.8 ns	2.0*	0.24 ns	29.2	1.4	0.24
2000–250	56.3*	2.8*	0.33*	26.7	1.5	0.22
250–53	48.9*	2.5*	0.29*	18.1	1.0	0.14
<53	27.7*	1.9*	0.19*	16.9	0.9	0.11
	(g kg^{-1} soil)			(g kg^{-1} soil)		
Whole soil	25.6*	1.5*	0.19*	11.7	0.8	0.12

Values followed by * are significantly different between undisturbed and cultivated sites within aggregate size ($p<0.05$), ns = not significant.

Table 3
Organic carbon bound to the mineral fraction (MOC) and particulate organic carbon (POC) contents in soil, small macroaggregates (2000–250 μm), microaggregates (250–53 μm) of cultivated and undisturbed sites

	(μm)	Undisturbed	Cultivated
		(OC g kg^{-1})	
MOC	2000–250	32.9*	19.1
POC	2000–250	27.5*	16.4
MOC	250–53	23.5*	7.3
POC	250–53	23.2*	1.4
		(OC g kg^{-1} soil)	
MOC	Soil	18.3*	9.6
POC	Soil	7.7*	1.8

Values followed by * are significantly different between undisturbed and cultivated sites within aggregate size ($p<0.05$), ns = not significant.

3.3. Mineral bound organic carbon (MOC) and particulate organic carbon (POC) contents

The total carbon concentration of the undisturbed soils was 26 g kg^{-1} (=18.3+7.7 g OC kg^{-1} of soil), and the POC content was 30% of the total carbon (Table 3). Cultivation reduced total organic carbon content to 11.4 g kg^{-1} (=9.6+1.8 g kg^{-1}), and 15% of this amount was POC. The data showed considerable differences in MOC and POC contents between undisturbed soil and cultivated soils. Cultivation appeared to have also harmfully affected the MOC and POC contents. The MOC content in the large macroaggregate (2000–250 μm) decreased from 32.9 g kg^{-1} in the undisturbed soil to 19.1 g kg^{-1} in the cultivated soil, which meant a 42% reduction as a result of cultivation. The decrease in MOC content was even more drastic in the microaggregate (250–53 μm) fraction, where a reduction of 69% was detected due to cultivation. Similar results were found by Hevia et al. (2003), for entic and typic Haplustoll soils of Argentina.

The organic fraction mostly affected by tillage was the POC in whole soils, where its content decreased from

Table 4
Humic acids (HA) and fulvic acids (FA) content (g kg^{-1}) in soil and the small macro- and microaggregate fractions of undisturbed and cultivated sites

Fraction	HA		FA	
	Undisturbed	Cultivated	Undisturbed	Cultivated
2000–250 μm	22.3*	8.6	2.6*	0.8
250–53 μm	19.4*	5.6	2.2*	0.7
Soil	12.8*	7.5	1.4*	0.6

Values followed by * are significantly different between undisturbed and cultivated sites within aggregate size ($p<0.05$).

7.70 g kg^{-1} in undisturbed soil to 1.83 g kg^{-1} in the cultivated soil, which means a 76% reduction due to cultivation. The loss of POC in the microaggregates (250–53 μm) was more dramatic, with the data showing a decrease of 94% due to cultivation, indicating an almost total loss of POC carbon in the organic core. This destruction of POC by microbial attack was not replaced by new organic matter, due to the exposure of POC as a result of destruction of macroaggregates (Six et al., 1999).

3.4. Humic acid (HA) and fulvic acid (FA) contents

The HA contents were substantially higher than those of FA in whole soils and in the aggregate fractions, which is common, though not the general rule, in mollisols (Tan, 1978). The HA and FA contents were reduced in the range of 40–70% in whole soils and the aggregate fractions. The lowest decrease of 41% was noted in the HA content of whole soil, where the content was reduced from 12.81 g kg^{-1} in the undisturbed soil to 7.49 g kg^{-1} in the cultivated soil (Table 4). The highest decreases of 68–71% data were detected for the HA content in the microaggregate (250–53 μm) fraction and FA contents in both the macro- and microaggregates. Humic substances, considered to be the permanent binding agents, have decreased by cultivation in the same percentage as transient and temporary binding agents. Although HS molecules are more recalcitrant than the other binding agents, their chemical recalcitrance does not account for the persistence of HS in soil if not their occlusion in the aggregates (Golchin et al., 1997). Once the aggregates are broken down by cultivation, the HS are exposed to microbial attack and are degraded.

4. Conclusions

The results discussed above indicate that the large macroaggregates (2800–2000 μm) were the most sensitive to the effect of cultivation. The decomposition of transient dilute acid soluble carbohydates (CHda), and hot water soluble carbohydates (CHhw), brought about by cultivation, was one of the reasons for a total breakdown of the macroaggregates into microaggregates. This has resulted into the exposure of exposing the POC, the inner organic core, making it subject to rapid microbial attack. An almost total loss of POC was observed, with a decrease of 94% of POC detected in the microaggregates.

The results also suggest that both HA and FA were affected by cultivation apparently in the same magnitude as the transient and temporary binding despite their intrinsic chemical recalcitrance.

For the soil used in this study, which is representative of the peanut growing area of Argentina, an aggregate hierarchy was observed. Cultivation produces the rupture of macroaggregates (2800–250 μm) rich in organic matter with a subsequent increase of microaggregates (250–53 μm) depleted in organic matter.

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