Revista Brasileira de Ciência do Solo

Division - Soil Use and Management | Commission - Soil and Water Management and Conservation

Long-Term Effect of Soil Use and Management on Organic Carbon and Aggregate Stability

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ABSTRACT: The conversion of native grassland into farmland causes changes in the soil. Tillage has profound effects on soil organic matter. The intensification of soil tillage decreases soil quality by reducing aggregate stability. Soil aggregate stability and soil organic matter are key indicators for soil quality and environmental sustainability in agro-ecosystems. The aim of the present study is to evaluate the total organic carbon content and the physical and chemical fractions of the organic matter in a soil under different uses and types of management over 27 years. Four soil tillage treatments with two annual crops were evaluated (no-tillage, NT; rotating tillage, RT; minimum tillage, MT; and conventional tillage, CT), as well as bare soil (BS) (standard plot of the Universal Soil Loss Equation - USLE) and natural grassland (NG) as a reference area. The experiment was carried out in an Inceptisol (*Cambissolos*) in southern Brazil. We determined total organic carbon (TOC) and particulate organic carbon (POC), and organic carbon associated with soil minerals (OCam). The chemical fractionation of carbon was into fulvic and humic acids, and humin. In addition, soil aggregates were divided into five size classes. The type of soil tillage affected the soil organic carbon content, namely TOC, POC, and OCam, as well as the composition of the physical and chemical fractions and their distribution in the arable soil layer. There was a positive relationship between stable aggregates and organic carbon in the soil: the higher the proportion of aggregates in class 1, the higher the organic carbon content. The results support the hypothesis that the carbon stock depends on intensification of a conservation tillage system with a continuous input of C through biomass, which maintains and supplies a continuous flow of C to the carbon transformation processes in the soil.

Keywords: equivalent mass, carbon stock, humic substance, aggregate classes.

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Received: December 13, 2017
Approved: April 26, 2018

How to cite: Wolschick NH, Barbosa FT, Bertol I, Bagio B, Kaufmann DS. Long-term effect of soil use and management on organic carbon and aggregate stability. Rev Bras Cienc Solo. 2018;42:e0170393.
https://doi.org/10.1590/18069657rbcs20170393

INTRODUCTION

Soil tillage has a profound effect on soil organic matter (SOM). Intensification of tillage decreases soil quality by reducing aggregate stability (Seben Junior et al., 2014). Soil aggregate stability and SOM are key indicators for soil quality and environmental sustainability in agro-ecosystems. Soil organic carbon (SOC) has been drastically reduced by conversion of natural vegetation to farmland. Conventional tillage (CT) was the predominant system applied in the early years after conversion. Currently, there are other types of soil tillage, e.g., no-tillage (NT).

There are significant differences in the SOC stocks and in the rate of SOC sequestration between CT and NT (Sá et al., 2015), the same authors noted that this difference was confined to the upper soil layers. In NT, carbon accumulation increased with an increase in the input of biomass-C. High SOC resilience indicates considerable potential for C accumulation under NT.

Compared to tillage that involves plowing, disking, or chiseling, no-tillage is a more conservationist type of soil management because it interferes little with soil structure. Furthermore, crop rotation and the amount and management of crop residues added by the crops involved in the cropping system may favor the accumulation of organic C by virtue of physical protection of the organic matter by the aggregates which they helped to form and in which the carbon is contained (Bayer et al., 2000).

No-tillage with a large addition of plant biomass to the soil enhances SOC storage. This constitutes an effective way to restore SOC over time (Hok et al., 2015). The SOC may be vertically distributed in deeper soil layers in long-term conservation agriculture in response to high biomass-C inputs from deep-rooting cover crops.

Soil organic matter fractions are the most sensitive way to detect changes in soil tillage over time (Rosset et al., 2016). No-tillage leads to greater carbon stability with a predominance of the humin fraction. Soil tillage and residue management affect the input of organic residues into the soil and, thus, its physicochemical properties, above all aggregate stability (Guimarães et al., 2013). Compared to NT, CT negatively affects soil aggregate stability, which leads to an increased susceptibility to slaking (Paul et al., 2013) and soil erosion (Bertol et al., 2014).

The adoption of an NT system improves soil aggregation and aggregate stability (Seben Junior et al., 2014). Stable aggregation has frequently been shown to reduce susceptibility to formation of runoff and water erosion (Bertol et al., 2014), depending on clay mineralogy. In addition, fresh residue inputs and active root growth led to more and stronger organic cementing in 2:1 than in 1:1 clay minerals in soils (Denef and Six, 2005).

An analysis by Heikkinen et al. (2013) revealed that the shift towards cultivation of annual crops in recent decades has contributed to C losses. Soil cultivation seems to bring about more of a decrease in soil carbon in coarser soils. In finer soils, with more silt and clay, a lot of carbon is contained in aggregates, where it is stabilized and protected.

Insufficient residue addition can affect soil aggregate stability and SOC. Residue on the surface without tillage did not affect SOC in the short term, but when residue was incorporated by tillage, SOC increased underground rather quickly (Paul et al., 2013). With no-tillage, it was observed that soil carbon accumulation occurs preferentially as particulate organic carbon (POC), which is more sensitive to decomposition as a consequence of tillage than total organic carbon (Bayer, 2003). Nowadays, further loss of SOC significantly increases the risk of erosion, and the SOC in many clayey soils may fall to the critical range for stable aggregates, calling for careful management of these fields to sustain their soil structure and productivity in the future (Soinne et al., 2016).

Soil tillage mainly influences the decomposable SOC pools, but it also affects more stable SOC fractions. The size fractionation of SOM can help assess the changes resulting from land use, because of different sensitivity of the various fractions to the effects of soil management (Bayer, 2003). The implementation of minimum tillage practices significantly increased the contents of humic acid (HA) and fulvic acid (FA) strongly bound to clay and of humic acid bound to calcium in the plowed layer (Liaudanskiene et al., 2013).

The ratio between the different humic substances present in a soil varies greatly among different soils, due to different clay contents. Higher C contents in the humin (H) fraction compared to the HA and FA fractions occur in soils with higher clay content (Rosset et al., 2016). This indicates a higher interaction of this recalcitrant SOM fraction with the clay fraction. In clay soils, the (HA+FA)/H ratio is less than unity. Chemical fractionation was used to evaluate the carbon content in the different humic fractions of the soil. The humic substances (humin, humic acid, and fulvic acid) represent the largest fractions of C present in a soil.

The number of studies with chemical and physical fractionation of SOM in soils with different uses and management over a long period is limited. To show the importance of soil management to maintain SOC, this study aimed to evaluate the total organic carbon content, the physical and chemical fractions of the organic matter, and the aggregate classes present in this soil under different soil tillage systems over a period of 27 years.

MATERIALS AND METHODS

The experimental site (27° 49’ S and 50° 20’ W, at an altitude of 923 m) was established in 1988 and is located in the municipality of Lages in the state of Santa Catarina in southern Brazil. The climate type according to the Köppen classification system (Alvares et al., 2013) is Cfb, i.e., subtropical, humid, without a dry season, with cool summers and frequent frosts in winter. The mean annual temperature is 15.7 °C and mean annual rainfall is 1,533 mm (Schick et al., 2014). The soil of the experiment site is a *Cambissolo Húmico Alumínico léptico* (Santos et al., 2013), which correspond to an Inceptisol according to the Soil Survey Staff (2014), with 180 g kg^{-1} sand, 420 g kg^{-1} silt, and 400 g kg^{-1} clay in the topsoil (0.00-0.40 m layer).

The experiments were conducted in experimental plots of 22.1 × 3.5 m (77.35 m^2). Four soil tillage treatments with two annual crops, as well as one bare soil were evaluated, with two replicates (plots) each: i) no-tillage (NT); ii) minimum tillage (MT, here one chiseling and two disking, layer of 0.00-0.17 m); iii) conventional tillage (CT, here one plowing and two disking, layer of 0.00-0.17 m layer); iv) rotating tillage (RT, i.e. NT, MT, and CT in sequence); v) bare soil with one plowing and two disking as in a standard plot for derivation of the Universal Soil Loss Equation (BS); and vi) natural grassland (NG) as a reference. In BS, the surface was kept free of vegetation and without a crust.

At the time the experiment was set up, the pH was 4.7. To adjust the pH to 6 (suitable for most annual crops), 12 Mg ha^{-1} of dolomitic limestone was incorporated per plot by two plowings and disking parallel to the slope to a depth of 0.17 m. In the year 1992, another 3.5 Mg ha^{-1} of dolomitic limestone per plot was incorporated as described above. In the year 2012, a new application of limestone was made on all cultivated plots, but not on the bare soil. The amounts needed to elevate the pH to 6 were specific to each plot and were determined by individual soil sampling in the 0.00-0.10 m layer, as recommended by CQFS-RS/SC (1995). Fertilization was performed according to crop needs to achieve high yield, as recommended in the manure and liming manual.

Crops grown in rows in the NT, RT, MT, and CT treatments were corn (*Zea mays* L.), dry edible beans (*Phaseolus vulgaris* L.), and soybeans [*Glycine max* (L.) Merrill], all sown with the aid of a manual planter. Also grown were black oat (*Avena strigosa* L.), vetch

(*Vicia sativa* L.), forage turnip (*Raphanus sativus* L.), and wheat (*Triticum aestivum* L.). They were broadcast by hand.

Soil samples were collected in March 2015 at the layers of 0.000-0.025, 0.025-0.050, 0.05-0.10, 0.10-0.20, and 0.20-0.40 m at two points in each experimental plot (four replicates per treatment). The samples were dried and sieved though a 2 mm mesh. Total organic carbon (TOC) was determined using the wet combustion method. Soil organic compounds were oxidized by dichromate in acidic compound, with application of external heat (150 °C for ~ 1 min) for complete oxidation of the compounds, as described in Tedesco et al. (1995). Particulate organic carbon (POC) was fractionated from a mixture of 20 g of soil and 60 mL of sodium hexametaphosphate (5 g L^{-1}) shaken for 16 h. After that, the suspension was passed through a 53 µm sieve (Cambardella and Elliot, 1993). After fractionation, the samples were dried at 60 °C, and then ground in porcelain vessels, followed by determination of carbon content by the method of Tedesco et al. (1995), cited above.

The stocks were calculated using equivalent mass (Ellert and Bettany, 1995). The TOC stocks (Mg ha^{-1}) were calculated taking into account the soil at the five layers studied. The POC stocks were calculated considering the mass of the particles retained in the sieve used to determine the POC content. The content and stocks of organic carbon associated with soil minerals (OCam) were calculated from the difference between TOC and POC. The equivalent mass method of the soil uses the soil mass of a treatment as a reference, which is taken as the basis for calculation of the stock in all other treatments. For the present study, we considered the soil masses of the corresponding layers of the natural grassland (NG) as reference, which represents the original condition of the soil.

Chemical fractionation of carbon was carried out using the methodology described by Benites et al. (2003), which is based on solubility in acidic and alkaline media. For this 20 mL of NaOH 0.1 mol L^{-1} was added to 1.5 g of soil. After 24 h, the samples were centrifuged at 2,500 rpm for 30 min. This step was repeated twice. In each process, the supernatant liquid was separated and the humin fraction subsequently determined in the precipitated fraction. The pH of the precipitated fraction was adjusted to 1.0 with the addition of an H_2SO_4 solution (20 %) and then left to rest for a period of 18 h. The precipitate was then passed through a 0.45 µm membrane filter under a vacuum, thus obtaining the fulvic acid fraction. To get the humic acid fraction, NaOH 0.1 mol L^{-1} was added to the precipitate trapped in the filter until it was completely washed out. After this procedure, the volumes of the fractions obtained (fulvic and humic acid) were adjusted to a common volume of 50 mL with distilled water. Subsequently, the organic carbon content in each fraction was determined by the wet combustion method.

In the laboratory, the field-moist soil samples were gently broken apart by hand, all visible plant material and roots were removed, and the soil samples were homogenized to determine aggregate stability. This was done by wet sieving of 25 g subsamples, which were pre-wetted for 10 min and then placed in vertical oscillation equipment for 10 min over a set of sieves of 4.76 (Class 1: 6.375 mm), 2.00 (Class 2: 3.375 mm), 1.00 (Class 3: 1.500 mm), and 0.25 mm (Class 4: 0.625 mm) mesh size. What passes through the last sieve yields Class 5 (0.125 mm). The percentage of sieved aggregates in each class was calculated for each sample as described by Kemper and Chepil (1965).

Statistical analysis of the data was performed using Assistat (Silva et al., 2016). Data normality was tested with the Shapiro-Wilk test. Those not following a normal distribution were log-transformed. Significant differences between the treatments and the reference area were determined from the Dunnett test. Subsequently, the Duncan test was carried out to establish whether the treatments were different or not ($p \leq 0.05$). The least significant difference (LSD) between treatment means comparing all depths evaluated is shown in table 1. The standard deviation (SD) at each layer for each treatment variable is shown in figures 1 and 2.

Table 1. Contents and stocks of total organic carbon (TOC), particulate organic carbon (POC), and organic carbon associated with minerals (OCam) in an Inseptisol after 27 years under various soil tillage methods: no-tillage (NT), rotating tillage (RT), minimum tillage (MT), bare soil (BS), conventional tillage (CT), and natural grassland (NG) as a reference

Treat.	Layer	Equivalent layer content				Equivalent mass of soil				
		Density	TOC	POC	OCam	Layer	Mass	TOC	POC	OCam
	m	Mg m^{-3}	g kg^{-1}			m	Mg ha^{-1}			
NT	0.000-0.025	1.02	108.9 a*	82.7 a*	26.2 a	0.000-0.019	196	21.3 a	16.21 a*	5.13 a*
	0.025-0.050	1.20	57.2 ab	31.0 ab	26.1 ab	0.019-0.044	298	17.0 ab	9.26 ab*	7.8
	0.050-0.100	1.35	51.7 a	15.7 c*	35.9 a	0.044-0.093	661	34.1 a	10.44 c*	23.75
	0.100-0.200	1.33	47.1 a	5.1 cd*	41.9 a	0.093-0.196	1366	64.3 a	7.05 c*	57.31
	0.200-0.400	1.29	43.4 a	5.3 ab*	38.1 a	0.196-0.446	3205	139.2 a	17.04 b*	122.20
Total						0.000-0.446		274.72	57.51	217.21
RT	0.000-0.025	0.99	55.4 bc*	41.9 b*	13.5 b	0.000-0.020	196	10.8 bc*	8.2 b*	2.6 bc
	0.025-0.050	1.09	55.0 a	32.7 ab	22.3 b	0.020-0.047	298	16.4 ab	9.7 ab	6.6
	0.050-0.100	1.14	46.5 a	23.0 b	23.5 b	0.047-0.105	661	30.8 a	15.2 b	15.5
	0.100-0.200	1.26	46.2 a	10.7 bc*	35.5 ab	0.105-0.213	1366	63.2 a	14.7 a	48.4
	0.200-0.400	1.30	37.7 a	5.6 ab*	32.1 ab	0.213-0.460	3205	120.9 a	18.0 b*	102.9
Total						0.000-0.460		242.24	65.98	176.26
MT	0.000-0.025	0.99	65.1 b*	51.1 b*	14.0 b	0.000-0.020	196	12.7 b*	10.0 b*	2.7 bc
	0.025-0.050	1.08	60.9 a	42.0 a	18.9 b	0.020-0.048	298	18.1 a	12.5 a	5.6
	0.050-0.100	1.26	53.3 a	29.4 a	23.9 b	0.048-0.101	661	35.2 a	19.4 a	15.8
	0.100-0.200	1.39	41.8 a	7.1 bc*	34.7 ab	0.101-0.200	1366	57.2 a	9.6 bc*	47.5
	0.200-0.400	1.39	40.9 a	5.2 ab*	35.6 a	0.200-0.431	3205	131.0 a	16.8 b*	114.1
Total						0.000-0.431		254.48	68.59	185.90
BS	0.000-0.025	1.17	27.7 d*	2.5 d*	25.2 ab	0.000-0.017	196	5.4 d*	0.4 d*	4.9 ab*
	0.025-0.050	1.25	30.9 b	2.4 c*	28.5 a	0.017-0.041	298	9.2 c*	0.7 c*	8.4
	0.050-0.100	1.30	33.2 b*	2.1 d*	31.0 ab	0.041-0.092	661	21.9 b*	1.4 d*	20.5
	0.100-0.200	1.43	29.3 b*	2.2 d*	27.1 b	0.092-0.188	1366	40.1 b*	3.0 d*	37.1
	0.200-0.400	1.32	25.8 b*	1.9 b*	23.8 b	0.188-0.433	3205	82.7 b*	6.1 b*	76.5
Total						0.000-0.433		159.52	11.86	147.66
CT	0.000-0.025	1.11	43.1 c*	19.9 c*	23.2 ab	0.000-0.018	196	8.4 c*	3.9 c*	4.5 ab
	0.025-0.050	1.14	48.5 ab	20.2 b*	28.2 a	0.026-0.044	298	14.4 b	6.0 b*	8.4
	0.050-0.100	1.30	43.3 ab	13.2 c	30.0 ab	0.044-0.095	661	28.6 ab	8.7 c	19.9
	0.100-0.200	1.37	39.9 a	9.5 ab*	30.3 b	0.095-0.195	1366	54.5 a	13.1 ab*	41.4
	0.200-0.400	1.35	41.5 a	9.6 a	31.9 ab	0.195-0.433	3205	133.1 a	30.8 a	102.3
Total						0.000-0.433		239.31	62.65	176.66
NG	0.000-0.025	0.79	133.34	121.20	12.14	0.000-0.025	196	26.13	23.76	2.38
	0.025-0.050	1.19	61.29	41.37	19.92	0.025-0.050	298	18.26	12.32	5.93
	0.050-0.100	1.32	51.43	21.50	29.93	0.050-0.100	661	33.99	14.21	19.79
	0.100-0.200	1.37	43.49	14.10	29.39	0.100-0.200	1366	59.41	19.26	40.15
	0.200-0.400	1.60	40.36	10.89	29.47	0.200-0.400	3205	129.37	34.90	94.48
Total						0.000-0.400		266.45	104.50	162.42
SD	0.000-0.025	0.09	32.57	32.48	5.82			6.38	6.37	1.14
SD	0.025-0.050	0.06	8.39	11.30	3.64			2.50	3.37	1.08
SD	0.050-0.100	0.05	5.56	7.12	3.57			3.67	4.71	2.36
SD	0.100-0.200	0.04	4.46	3.33	4.22			6.10	4.55	5.77
SD	0.200-0.400	0.08	4.35	2.54	3.45			13.94	8.15	11.06

Note: Normality of the data by the Shapiro-Wilk test. We applied the Dunnett Test for comparison of the NG reference area with the treatments; mean values followed by (*) differ from the NG. Mean values followed by the same letter among the five treatments do not differ statistically from each other by the Duncan test at the 5 % probability level. SD = standard deviation at each layer for each treatment variable. Treat. = treatment. TOC (Tedesco et al., 1995), POC (Cambardella and Elliot, 1993), (TOC-POC = COam). Equivalent mass of soil and stocks of C (Ellert and Bettany, 1995).

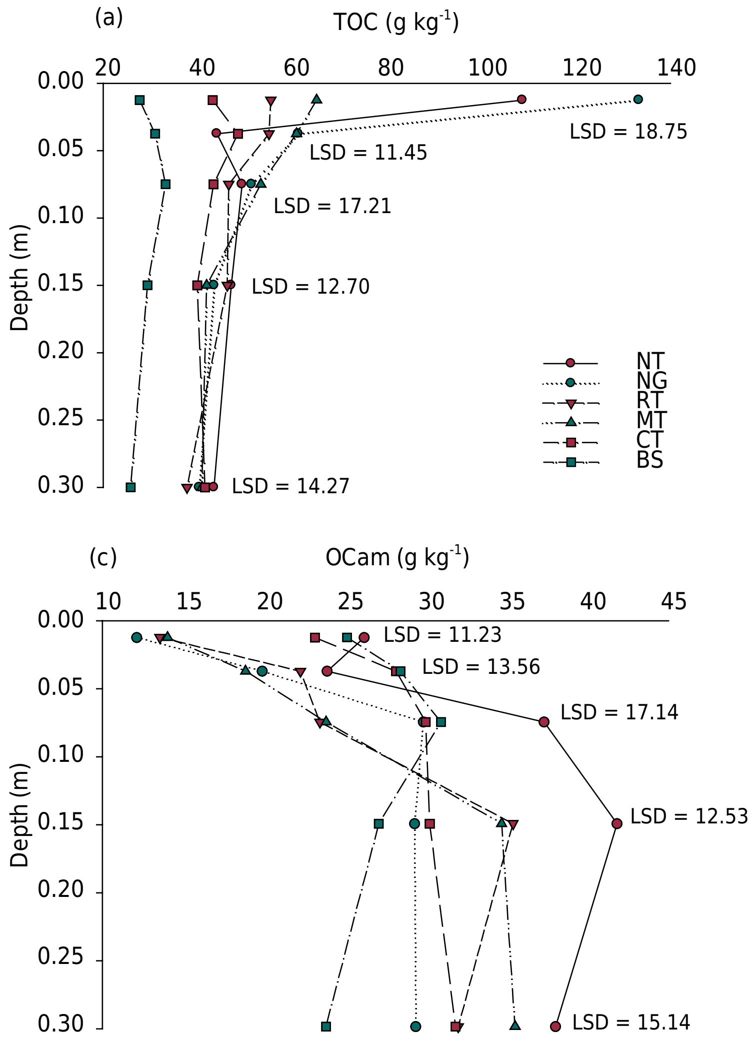

Figure 1. Contents of total organic carbon (a), particulate organic carbon (b), and organic carbon associated with minerals (c) in an Inceptisol after 27 years under various soil tillage methods: no-tillage (NT), rotating tillage (RT), minimum tillage (MT), conventional tillage (CT), bare soil (BS), and natural grassland (NG) as a reference, in the 0.000-0.025, 0.025-0.050, 0.050-0.100, 0.100-0.200, and 0.200-0.400 m soil layers. LSD = least significant difference between treatment means in the treatments comparing all depths evaluated; TOC = total organic carbon; POC = particulate organic carbon; OCam = organic carbon associated with minerals.

RESULTS AND DISCUSSIONS

In the top 0.025 m, a TOC loss of 18, 58, 51, 68, and 79 % was observed in the NT, RT, MT, CT, and BS treatments, respectively, compared to NG, which had the highest least significant difference (LSD) (Table 1 and Figure 1a). Despite these high LSD values, the differences among treatments were significant. The TOC losses in these treatments were dependent on the soil tillage the plots were subjected to after their conversion from NG to cropped soils with tillage or to bare soil with tillage but without a crop.

In the 0.000-0.025 m layer, the highest TOC levels were found in the reference area (NG), with significant differences from the other treatments. This can be explained by the greater stability of the soil in this treatment without agricultural use, resulting in a dynamic balance between the average rates of carbon addition to the soil and the rates of mineralization of the organic residue and losses in the form of CO_2. When comparing only the treatments (omitting NG), NT showed the highest TOC content by far. In NT, the absence of plowing and the accumulation of organic residues on the surface layer favor an increase in TOC in the surface layer (Rosa et al., 2008). In the long-term, SOC can be distributed vertically into deeper soil layers in response to high C inputs by cover crop biomass, and by deep rooting in conservation management (Hok et al., 2015).

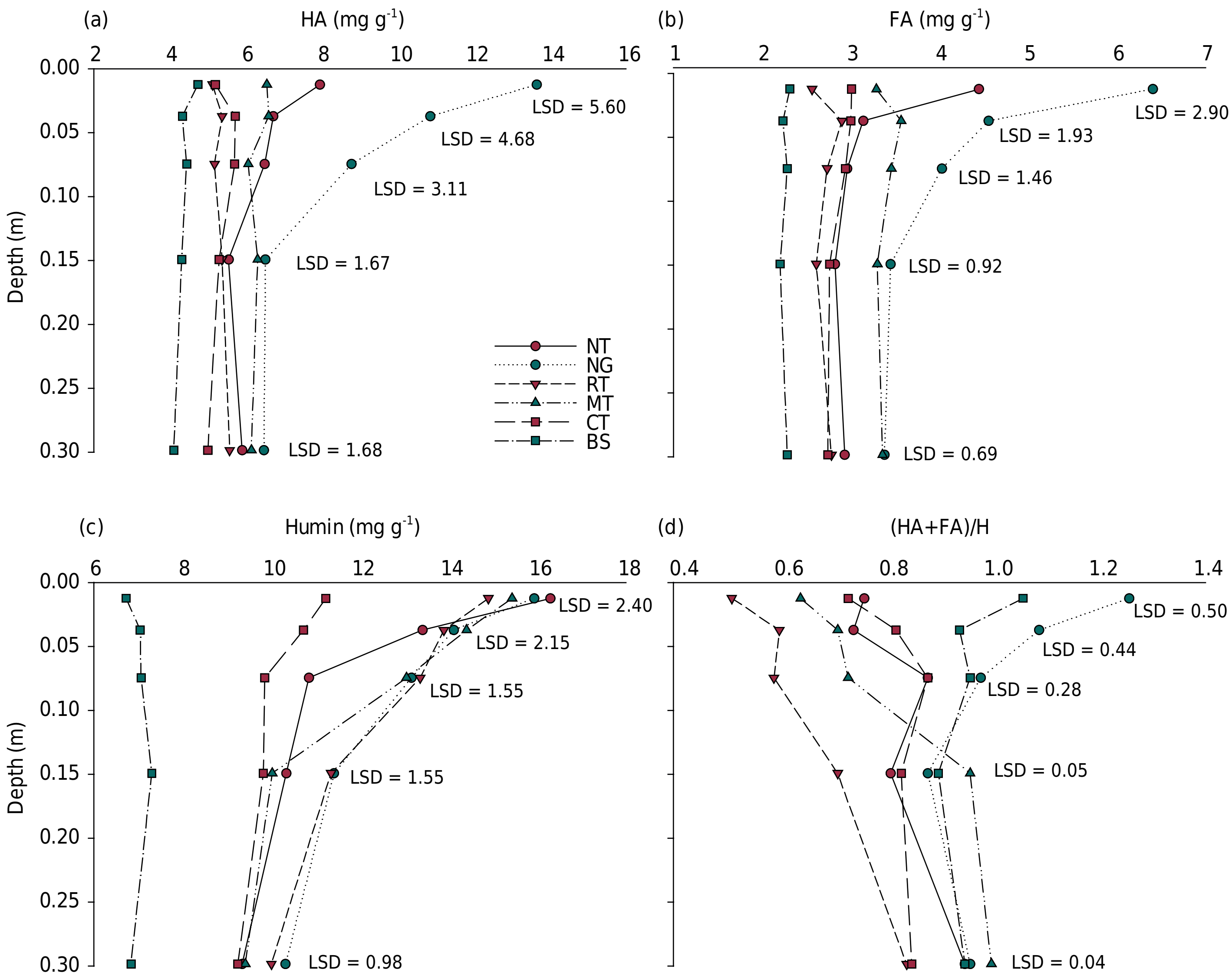

Figure 2. Contents of humic acid (a), fulvic acid (b), humin (c), and the (HA+FA)/H ratio (d) in an Inceptisol after 27 years under various soil tillage methods: no-tillage (NT), rotating tillage (RT), minimum tillage (MT), conventional tillage (CT), bare soil (BS), and natural grassland (NG) as a reference, in the 0.000-0.025, 0.025-0.050, 0.050-0.100, 0.100-0.200, and 0.200-0.400 m layer. LSD = least significant difference between treatment means in the treatments comparing all depths evaluated; HA = humic acid; FA = fulvic acid; H = humin.

The treatments RT, MT, and CT showed a reduction in the TOC contents in the 0.000-0.025 m layer compared to the NT, especially CT, which had 60 % less TOC than NT and 68 % less TOC than NG. This is a result of plowing and disking the soil twice a year and can be attributed mainly to the higher rate of mineralization of SOM, since soil tillage breaks down the aggregates and exposes the organic matter to microbial activity, leading to carbon loss in the form of CO_2 (Teixeira et al., 2011). In the BS treatment, the soil tillage was the same, except that there was no vegetation. This lack of vegetation at the soil surface and, therefore, the lack of C input in the soil for 27 years resulted in a lower TOC (only 28 g kg^{-1}) compared to the other plots. The influence of soil tillage on TOC seems to depend on the depth to which the residues are incorporated into the soil (Liaudanskiene et al., 2013). According to the same authors, minimum tillage (MT) leads to a higher organic carbon (OC) content in the surface layer (0.00-0.15 m), but in the layer below (0.15-0.25 m), CT does.

In the 0.025-0.050 m layer, the BS treatment had 50 % lower TOC compared to NG. In the other layers (0.05-0.10, 0.10-0.20, and 0.20-0.40 m), TOC in the BS treatment remained statistically lower than in the other soil tillage treatments. Even the organic carbon stored in the fine fractions of the soil is subject to microbial decomposition because of the intense mobilization of the soil and the low capacity of protection of the clay minerals present in an Inceptisol (Stürmer et al., 2007). Management systems without soil turnover or with less soil turnover tend to store more OC (Rangel and Silva, 2007).

The NG had the highest POC content in the 0.000-0.025 m layer (Figure 1b). Among the treatments, NT was superior by 50 % in relation to the MT and 38 % in relation to the RT conservation treatments. The POC in NT was 32 times higher than in BS and 4 times higher than in CT.

In the 0.025-0.050 m layer, CT differed statistically from NG and the other treatments. In the 0.05-0.10 m layer, NT and BS differed from the reference area. In the NT treatment, there was a 50 % reduction in POC in relation to the reference area; in the BS, it was 10 times lower than in the reference area. In an Inceptisol, Stürmer et al. (2007) observed a 25 % reduction in POC in the 15 years since conversion from native forest to CT. In our case (conversion of grassland to cropland), the reduction was 16.5 % in 27 years.

In the 0.10-0.20 m layer, only the RT treatment did not differ statistically from NG, and in the 0.20-0.40 m layer, CT did not differ from NG. These results show the effect of soil turnover and incorporation of plant residues into the soil after the conversion from natural vegetation to agricultural crops, and the concurrent reduction in organic matter input into the soil by the crops. The increase in carbon losses is a consequence of the oxidation of organic matter. However, an alteration in the soil environment can be a limiting factor for microbial activity, which reduces the decomposition rate of organic matter (Passos et al., 2007). Soil management with the use of a plow reduces the C contents in the soil. This plow action tends to homogenize the soil to a certain depth, unlike NT, where the residues accumulate on the surface (Liaudanskiene et al., 2013).

The OCam in the treatments did not differ statistically from NG at any of the depths evaluated (Figure 1c). Among the treatments, NT stood out with the highest OCam content, except in the 0.025-0.050 m layer. However, in this layer there was no statistical difference among the treatments. There are some points to be observed in relation to the physical fractionation of C and their proportions. In the surface layer (0.000-0.025 m), NG had 9 % of the TOC in the form of OCam, while in the NT treatment, it was 24 %. In the BS treatment, 91 % of the TOC was associated with minerals. This was the case at all depths evaluated. In general, we observed that the CT treatment had higher values, especially of the more resilient fractions of SOM.

Soil has a high capacity to protect organic matter (OM). High contents of clay and silt lead to stable aggregates and thus maintain a higher stock of OCam, which results in good physical condition of this soil. This fraction is less sensitive to changes, because it consists of organic material with strong bonds with the mineral fraction (Bayer and Bertol, 1999; Bayer, 2003). The OCam is in an advanced stage of humification, because of interaction with the mineral fraction and physical protection by stable micro-aggregates in the soil. It is also intrinsically resilient, due to its chemical stability.

The stocks of TOC, POC, and OCam in the equivalent soil mass are displayed in table 1. There are some differences in the surface layer (0.000-0.025 m), where NG differed statistically from the other treatments, except NT. Intensive soil cropping such as NT, with higher soil C additions through large biomass input, provides greater SOC storage (Hok et al., 2015). This is an effective way to restore SOC stocks over time. The lowest stocks among the treatments were found in the BS treatment, which were 3.9 times lower than NT for TOC, and 33 times lower than NT for POC. In the other layers, only the BS treatment differed statistically from NG. The C sequestration in NT soil is enhanced by high input cropping systems. No-tillage influences organic matter stabilization by reducing the decomposition rate, but on the other hand, improvements in the cropping system to further increase C addition may have a greater potential for soil C stock enhancement (Conceição et al., 2014). The OCam differed statistically in the first layer, in which BS and NT differed from NG.

In all treatments and layers evaluated, the largest stocks of C in the chemical fractions of OM were found in the humin fraction (Figure 2). The LSD values evaluated in this figure were lower, indicating better experimental accuracy. They ranged from 16.34 mg g^{-1} soil in the surface layer (0.000-0.025 m) of the NT treatment to 6.73 mg g^{-1} soil in the same layer in the BS treatment. This is a 59 % reduction in the C content in the form of humin in the BS treatment and reflects the influence of soil use and management. The humin fraction is less prone to changes due to soil management practices, since it is the most stable and resilient fraction. No-tillage implies greater carbon stability, with a predominance of the humin fraction (Rosset et al., 2016).

Higher content of C in the humic fractions of the soil surface layer could be related to intense microbial activity and, consequently, a higher rate of SOM decomposition (Guimarães et al., 2013). Carbon in the form of humin constitutes an important part of the TOC and is closely associated with the mineral fraction of the soil (Moraes et al., 2011). In general, we observed that the areas of natural pasture and conservation systems had higher values, especially of the more stable fractions of organic carbon.

The C contents in the HA and FA fractions tend to decrease with depth, which reflects reduction in the amount of crop residues in the subsurface soil compared to the surface layer (Figures 2a and 2b). This was most obvious in the NT and NG treatments, with a reduction of 26 % in NT and 53 % in NG for HA, and 34 % in NT and 43 % in NG for FA in the 0.000-0.025 m layer compared to the 0.200-0.400 m layer. In the RT, MT, and CT treatments, the values remained similar with depth. The use of minimal tillage practices in Inceptisols causes a significant increase in the content of humic and fulvic acids strongly linked with soil clay minerals and calcium-bound humic acids throughout the arable layer (Liaudanskiene et al., 2013).

The HA levels range from 13.70 mg g^{-1} for the 0.000-0.025 m layer in NG to 4.11 mg g^{-1} in the 0.20-0.40 m layer in the BS treatment. The levels of FA range from 6.43 mg g^{-1} in the surface layer (0.000-0.025 m) of NG to 2.21 mg g^{-1} in the 0.10-0.20 m layer of the BS treatment.

In the 0.000-0.025, 0.025-0.050, and 0.050-0.100 m layers, the NG area differed statistically from the other treatments, with the highest levels of HA and FA, except for the MT treatment in the 0.025-0.050 and 0.050-0.100 m layers. Among the treatments, BS was inferior to NT and MT. In the other layers, only BS was inferior to the reference area. The chemical fractions of OM are altered rather quickly in response to the management system adopted (Rosset et al., 2016).

Averaged across the layers studied, the C content in the form of humin was 58 % higher in NG than in BS (Figure 2c). In both treatments, the resilient C form was lower than the more labile forms. This was reflected in the (HA+FA)/H ratio. This ratio did not show a significant difference between the BS treatment and NG for any of the depths (Figure 2d).

The HA/FA ratio was not statistically different between the NG and the other treatments, nor among the treatments. The values ranged from 1.72 to 2.39, with an average value of 2 for the treatments and depths evaluated. This is evidence of the humic character of the SOM (Moraes et al., 2011). The same authors explain that the predominance of the HA fraction may be related to the synthesis of more complex structures from a structural rearrangement of less dense molecules, such as FA. The HA represents the intermediate fraction in the stabilization process of the humic compounds. These acids are therefore a natural marker of the humification process and reflect soil use and management (Rangel and Silva, 2007).

The distribution of the soil aggregate classes exhibits two distinct behaviors. The soil conservation systems have about 80 % of the aggregates in class 1 (6.375 mm) and about 15 % in class 2 (3.375 mm) (Figure 3). In contrast, the BS and CT treatments have a more uniform distribution across all aggregate classes, differing statistically from the

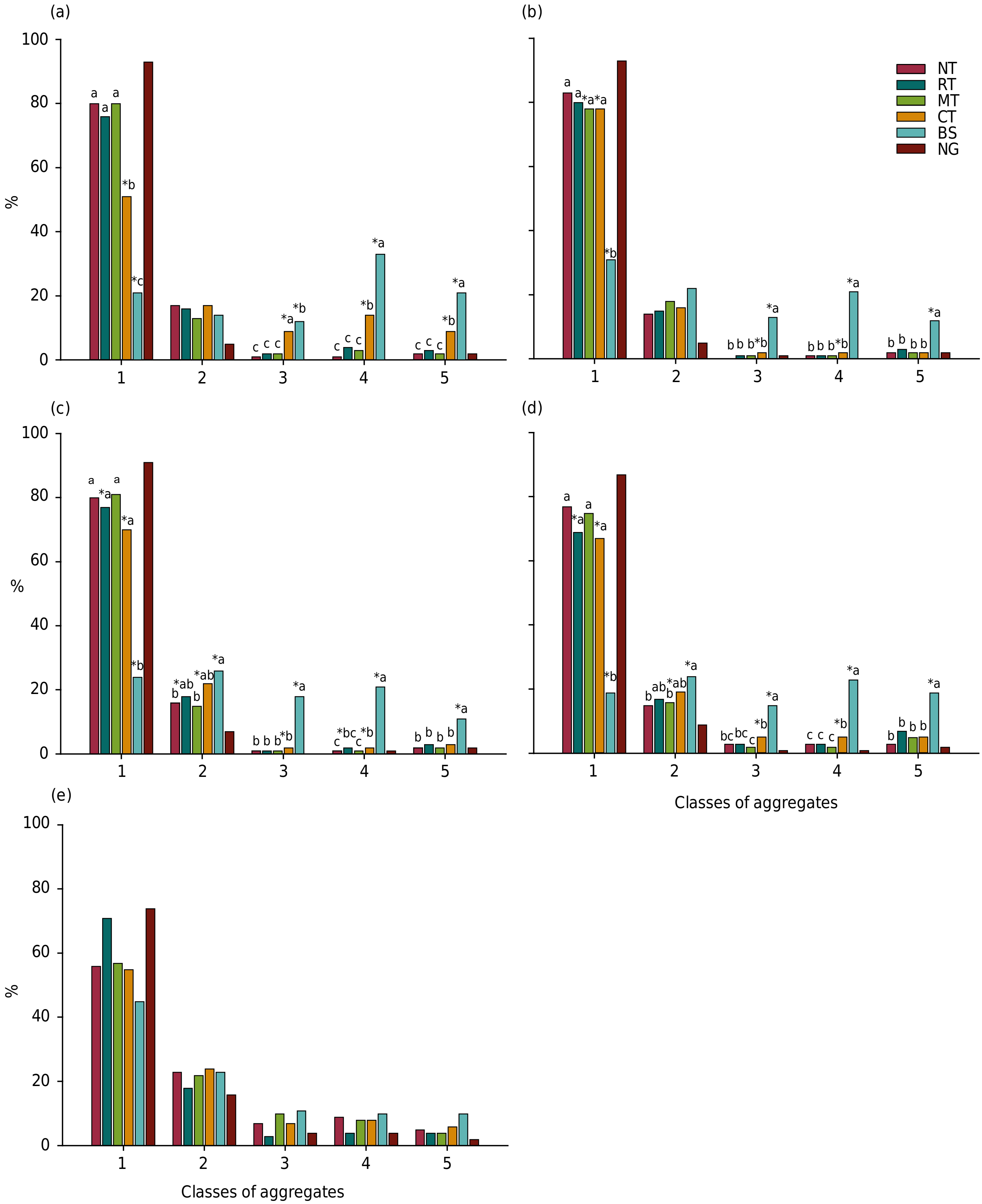

Figure 3. Distribution of the soil aggregates across 5 size classes (class 1: 6.375 mm; class 2: 3.375 mm; class 3: 1.500 mm; class 4: 0.625 mm; and class 5: 0.125 mm) at depths of 0.000-0.025 m (a), 0.025-0.050 m (b), 0.05-0.10 m (c), 0.10-0.20 m (d), and 0.20-0.40 m (e) in an Inceptisol after 27 years under various soil tillage methods: no-tillage (NT), rotating tillage (RT), minimum tillage (MT), conventional tillage (CT), bare soil (BS), and natural grassland (NG) as a reference. Note: Normality of the data by the Shapiro-Wilk test. The averages followed by (*) and/or by the same letter do not differ statistically from each other. We applied the Dunnett Test for comparison of the NG reference area with the treatments, and the Duncan Test for comparison among the five treatments at the 5 % probability level.

reference area as well as amongst themselves. In BS, the percentage of aggregates in the 5 classes ranges from 11 to 33 %, with the lowest percentage in the 0.050-0.100 m layer in class 5, and the highest in the surface layer in class 4. There is a significant difference between the CT and BS treatments, except in class 2 in the 0.00-0.200 m layer. The CT contains more than 80 % of the aggregates in classes 1 and 2. Results from Hontoria et al. (2016) show that properties related to SOM seem to play an important role in the aggregation of these soils: the higher the contents of TOC and POC, the higher the aggregate stability. When organic matter is oxidized, a substantial reduction in macro-aggregation occurs.

The 0.200-0.400 m layer showed no significant difference between the NG and the other treatments (Figure 3e). Likewise, the treatments did not show significant differences among themselves. This layer was not affected by any of the various types of tillage tested in our study because the plowing depth was only about 0.17 m. Hence, the soil structure in this layer remained unchanged.

CONCLUSIONS

The types of soil tillage affected soil organic carbon content and carbon stocks, as well as the composition of the physical and chemical carbon fractions and their distribution in the arable soil layer.

The largest proportions of the most labile (particulate organic carbon, acid humic, and acid fulvic) forms of soil organic matter were found in conservation tillage (no tillage, rotation tillage, and minimum tillage), and the highest proportion of the most recalcitrant (organic carbon associated of mineral and Humin) forms was found in conventional tillage. In our case, the bare soil treatment, in which the soil was kept free of vegetation, was the most disturbed and degraded system. The remaining carbon is associated with minerals. Regardless of the type of soil tillage, the humic substances represented the largest fraction of the organic matter in the Inceptisol studied here.

In the surface layer (0.000-0.025 m), there was a positive relationship between stable aggregates and organic carbon in the soil: the higher the organic carbon content, the higher the proportion of aggregates in class 1. In contrast, the lower the organic carbon content, the more evenly distributed the aggregates were among all classes. This behavior reflects the influence of the type of soil tillage.

ACKNOWLEDGMENTS

We thank the Capes, CNPq, and Udesc-CAV for their financial support, and Professor Dr. Heinz Borg for contributing to this paper.

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