Journal of Agriculture and Crops
ISSN(e): 2412-6381, ISSN(p): 2413-886X
Vol. 1, No. 6, pp: 75-82, 2015
URL: http://arpgweb.com/?ic=journal&journal=14&info=aims

Correction Equations for Wet Combustion Carbon Determination at Different Depths and Management Systems of a Rhodic Hapludox

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Abstract: Measurement of soil carbon is the focus of attention of present and future international conventions and agreements, related to global climate change. Past inventories and current carbon stock inventories involve different analytical methods, and methodological biases and uncertainties should be reduced to develop reliable estimates of the effects of land uses changes on total organic carbon. Furthermore, the carbon-equivalent is highly variable, and there is the need of using a specific correction factor for each location, resulting from the combination of land use, textural gradients, and sampling depth. In this context, the aims of this study were creating correction equations for the determinations through wet combustion (Walkley-Black- WB) for a Rhodic Hapludox based on the determinations made through dry combustion (CS) at different depths and management systems. The experimental design was 4 x 5 factorial with 3 replications. Treatments were: Conventional Tillage (CT); Minimum Tillage (MT); No-till with chisel plowing (NTC) and No-Till (NT). The collection depths were: 0-2.5; 2.5-5; 5-10; 10-20 and 20-40 cm. The measured carbon equivalent values ranged from 1.06 to 1.18 and were dependent on land use and soil depth. Rhodic Hapludox under different management presented the following order of carbon equivalent values: NTC < CT < NT < MT. The carbon equivalent values increased with depth. The high ratio between C-WB and C-CS (R2= 0.75, p= 0.0001) justifies the use of correction factors.

Keywords: Dry combustion; Walkley-Black; Correction factor.

1. Introduction

Measurement of soil carbon is the focus of attention of present and future international conventions and agreements, related to global climate change [1]. However, more efficient soil carbon quantification systems are necessary to offer better estimates of carbon determination [2].

Knowledge of their content is fundamental in diverse areas of soil science and various methods have been used for such, like muffle furnace gravimetric analysis [3], colorimetric method [4], laser induced breakdown spectroscopy [5, 6], mass spectroscopy [7], wet combustion [8] and dry oxidation.

The method for determination of Carbon through dry combustion stands out, due to its high precision and accuracy of results [4, 9].

In this method, a finely ground soil sample is placed in a capsule of Pt, being oxidized with O_2 at a temperature of approximately 950 °C, for 2 min in a combustion tube. After combustion, a flow of He is initiated and the CO_2, H_2O and N_2 produced by the combustion are passed by CuO/silver vanadate, to convert CO into CO_2 and remove S and halogen gases. The use of automatic element analyzers brought considerable advances, allowing quick and reliable analyses; however, the cost of each analysis and equipment maintenance is high. The methodology of quantification of TOC - "Total Organic Carbon" is a rapid analysis, highly reproducible and easily automated. However, its high cost often makes its execution unviable, for only a few laboratories in Brazil have the structure and capital to perform such analyses.

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Because of its practicality, the Walkley and Black [8] method has been more used in research and routine laboratory analyses. The most common analytical procedure for soil carbon determination is based on the oxidation of organic matter to CO_2 by dichromate ions, in a highly acidic medium. It is also denominated as wet determination or dichromate determination. In this method, a very strong oxidant is used, in this case potassium dichromate ($K_2Cr_2O_7$), in an acid solution for quantitative oxidation of carbon. This is a quick, highly reproducible and easily automated analysis. However, it presents analytical and environmental problems, due to the use of chrome.

Past inventories and current carbon stock inventories involve different analytical methods, and methodological biases and uncertainties should be reduced to develop reliable estimates of the effects of land uses changes on TOC. Furthermore, the carbon-equivalent is highly variable, and there is the need of using a specific correction factor for each location, resulting from the combination of land use, textural gradients, and sampling depth. In this context, the aims of this study were creating correction equations for the determinations through wet combustion (Walkley-Black) for Rhodic Hapludox based on the determinations made through dry combustion at different depths and management systems.

2. Material and Methods

2.1. Location, Climate and Description of the Area of Study

The present study was developed in an experiment on soil management systems established in 1988/89. Located at the experimental station of the Fundação ABC (ABC Foundation) in Ponta Grossa (PR), on the second plateau, Central East Mesoregion of the State of Parana, whose referential geographic coordinates are: 25°20'S and 50º20'W.? The altitude in the experimental area is 910 meters above sea level, and the climate is characterized as humid subtropical of the Cfb type (Köppen classification), with cool summers and frost in the winter. The average annual rainfall and temperature are 1,545 mm and 20°C respectively. The soil was classified as Rhodic Hapludox according to the soil taxonomy [10] and "Latossolo Vermelho eutrófico típico" by the Brazilian soil classification [11], clayey texture, deep, very well structured and drained, derived from reworked sandstone material from the Furnas formation and shales of the Ponta Grossa formation in the Devonian period [12]. The relief is slightly rolling with 2 to 7% slopes. A detailed description of the chemical and particle size analysis for this site is presented in Table 2.

2.2. Experimental Design and Statistical Analysis

The experimental design was a 4x5 Factorial with 3 replications, where we had 4 treatments and 5 collection depths. The treatments adopted were: 1) Conventional Tillage (CT) – performed through plowing to reach 20 cm depth, followed by two diskings for leveling; 2) Minimum Tillage (MT) – performed through chisel plowing to reach 30 cm depth followed by two diskings for leveling; 3) No-till with chisel plowing (NTC) – direct sowing over the crop residues maintained on the soil surface associated with chisel plowing to reach a depth of 30 cm every three years and 4) No till (NT) – direct sowing performed on crop residues maintained on the soil surface. The collection depths were: 0-2.5; 2.5-5; 5-10; 10-20 and 20-40 cm.

2.3. Walkley-Black Methods

The wet quantification of TOC (total organic carbon) (Walkley-Black) was performed from samples of 0.5 g of air-dried fine earth fraction (TFSA), ground in a mortar and passed through a 0.2mm screen sieve. They were transferred to a 500 mL Erlenmeyer flask, adding 10 mL of the $K_2Cr_20_7$ 0,167 mol L^{-1} solution and 20 mL of concentrated H_2SO_4; the Erlenmeyer flask was agitated for approximately one minute to promote the mixture of soil with the reagents, remaining at rest for 30 minutes. After this interval, 150 mL of distilled water was added and the solution was filtered in rapid filter paper, using a vacuum pump to accelerate the process. Then 50 mL of distilled water was added over the filter paper for complete removal of the solution. After filtering, 10 mL of concentrated H_3PO_4 and 10 mL of the diphenylamine indicator were added. Titration with $FeSO_4.7H_2O$ 0,25 mol L-1 was then performed, until the change from the color violet to green. In a parallel way, the same procedure was repeated for three control samples, without soil ("white").

2.4. Dry Combustion Methods

The quantification of TOC via dry combustion (CS) was undertaken from samples of 1.0 g air-dried fine earth fraction (TFSA), which were ground in degree and passed through a 0.2 mm screen sieve. Then they were individually transferred to a small tube and placed in a dessicator, for removal of possible existing moisture, before proceeding to analysis through dry combustion (CS) in a Truspec CN Analyser device, LECO brand, model SR-412. After the determinations, a correction factor among the methods was calculated, with the CS method being considered as a reference.

The results were submitted to analysis of variance by SISVAR 5.0, using the Tukey test at 5% probability. For obtaining response curves, the regression analysis procedure was used by the program JMP IN version 3.2.1 [13], using the F test at 5, 1 and 0.1% probability.

3. Results

The variables TOC (WB) and TOC (CS) presented significant interaction among treatments (management systems) x depth (Table 1).

The mean carbon value determined by CS was 7.2, 10.0 and 12.7% higher than the carbon amount determined by WB for the treatments CT, NT and MT, respectively (Table 3).

The Walkley-Black method, if compared to CS, showed values which are near TOC (not differing statistically) in surface layers (0–2.5 cm for CT and 2.5–5 cm for NT), with high values of TOC (Table 3). Nevertheless, it presented statistically different values in the samples from the layer from 2.5-5 and 5-10 cm for CT, 10-20 and 20-40 cm for NT, and 5-10 and 10-20 cm for MT, with low TOC values in comparison to soil surface.

The average deviation was 3.81 and 2.37 for WB and CS respectively for the CT treatment, whereas for the NTC treatment, the values were 4.46 and 0.76 for WB and CS respectively. For the MT treatment, the values were 3.93 and 1.18 for WB and CS respectively. However, for the NT treatment, the values were 1.11 and 1.39 for WB and CS respectively (Table 3). In the general average, the standard deviation was 3.33 and 1.57 for WB and CS respectively.

In this way, despite the soil management system used, the variation in WB was 112.1% higher than in CS. However, considering management system with some level of soil disturbance (CT, MT and NTC) the WB variation was 148% higher than the CS.

The correction factors of total organic carbon (Walkley-Black) for TOC (LECO – dry combustion) were 1.09; 1.17; 1.05 and 1.11 for CT, MT, NTC and NT respectively.

The correction equations suggested to be applied in accordance with the management systems on the results of C obtained by Walkley-Black are: C-CS = 1.83 + 1.01 C-WB; $R^2 = 0.99$*** (Figure 1a); C-CS = 8.55 + 0.64 C-WB; $R^2 = 0.65$* (Figure 1b); C-CS = 3.52 + 0.91 C-WB; $R^2 = 0.98$*** (Figure 1c) and C-CS = 4.87 + 0.94; $R^2 = 0.99$*** (Figure 1d), for CT; MT; NTC and NT respectively.

The correction equations suggested to be applied in accordance with the soil depths on the results of C obtained by Walkley-Black are: C-CS = 4.96 + 0.92 C-WB; $R^2 = 0.86$*** (Figure 2a); C-CS = 19.07 + 0.53 C-WB; $R^2 = 0.44$*** (Figure 2b); C-CS = 29.24 + 0.17 C-WB; $R^2 = 0.07$ (Figure 2c), C-CS = 26.48 + 0.11 C-WB; $R^2 = 0.05$ (Figure 2d) and C-CS = 9.32 + 0.72 C-WB; $R^2 = 0.58$*** (Figure 2e), for 0- 2.5; 2.5- 5.0; 5.0- 10.0; 10- 20 and 20-40 cm respectively. One notes that the ratio is significant between C-WB and C-CS in the layers of 0- 2.5; 2.5- 5.0 and 20- 40 cm, possibly for the reason of the standard deviation of this data having been very near between C-CS and C-WB if compared to the others (5.0-10.0 and 10- 20 cm), where the standard deviation was much greater for C-WB if compared to C-CS, with a significant ratio thus not occurring between these parameters (Table 4).

Ultimately, the high ratio between C-WB and C-CS ($R^2 = 0.75$***) justifies the use of the correction factors (Figure 3).

4. Discussion

The average value of carbon determined through CS was statistically greater than the value of carbon determined through WB for the treatments of CT, MT and NT. This highlights the greater efficiency in determination of carbon through CS compared to WB and makes it clear that the Walkley-Black method is not capable of oxidizing the soil carbon forms that are found more protected or in compounds with the mineral fraction of the soil, there being only partial oxidation of the OM. This data was ratified by Segnini, *et al.* [14] and Gatto, *et al.* [15]. According to Gatto, *et al.* [15] dry combustion, which occurs at a temperature of 925 °C, oxidizes all the C (organic and inorganic forms) of the sample, whereas in the wet combustion method (Walkley-Black), the oxidation of C, with or without heating, is not complete.Therefore, for the treatment of NTC, they were not sign, although one observes a trend for an increase in the average of carbon for the soil depths of 5-10 and 10-20 cm (Table 3).

Analyzing the surface depth (0-2.5 cm) of the management system with some soil disturbance level (CT, MT and NTC) we observed the C level was statistically equal in the both methods evaluated. This result could be explained by greater soil heterogeneity generated by frequent chisel plow and/or combined with disking operations. The effect of chisel causes a vertical disruption of the soil resulting in the formation of large clods, while the disking operation promotes a horizontal disruption of macroaggregates enhancing the oxidation process mainly at soil surface layers. In contrast, greater homogeneity is generally observed under NT soils due to the preservation of soil structure.

The Walkley-Black method, if compared to CS, showed values which are near TOC in surface layers (0–2.5 cm for CT and 2.5–5 cm for NT), with high values of TOC (Table 3). This data confirms the data obtained by Rheinheimer, *et al.* [16], where the authors suggest that the Walkley-Black method may be more precise for higher TOC content.

In other words, the variation was much greater for WB determination than for CS (Table 3), thus showing the greater efficiency in determination through CS for determination of C, this being due to possible incomplete oxidation which occurred in the determination through Walkley-Black [4]. This greater efficiency in determination of carbon was also verified by Segnini, *et al.* [14], Dieckow, *et al.* [17] in subtropical soils

Note that the correction factor follows the following scale: CT<NT. This result could be linked to the fact that soils managed under conventional tillage experience increased oxidizing conditions and exhibit higher annual rates of C loss through mineralization. In other words, the recalcitrant carbon, which is partially determined in acid combustion is found in greater exposure under CT area; thus, it has its determination facilitated in this method.

However, in the area of NT, where a greater quantity of recalcitrant carbon is found, a higher efficiency of the CS in relation to WB is found because in this case, the acid combustion is not facilitated by the exposure of the recalcitrant carbon through the management system. In other words, this strongly acid environment is not capable of oxidizing the forms of soil carbon that are found more protected or in compounds with the mineral fraction of the soil, with only partial oxidation of the OM occurring.

In contrast, dry combustion, through high temperature, is more complete for determination of this carbon. This fact is shown by the greater variation in the determination made by the Walkley-Black method, showing that determinations made by dry combustion may be more reliable.

This high ratio between C-WB and C-CS in all treatments (management systems) analyzed justifies the use of the correction factors (Figure 1). This data ratifies that observed by Dieckow, *et al.* [17] and Lettens, *et al.* [18] where the authors observed a strict correlation between the results of C Walkley-Black and the methods of dry combustion.

As reported by Ulmer, *et al.* [19], the equivalent values presented an increasing tendency with the soil depth, being higher at 20-40 cm depth in CT, MT and NT. This increasing Carbon equivalent in subsoil layers could be attributed to the proportion of chemically stabilized C and mineral-associated C [20, 21].

5. Conclusion

The measured carbon equivalent values ranged from 1.06 to 1.18 and were dependent on land use and soil depth. Rhodic Hapludox under different management presented the following order of carbon equivalent values: NTC < CT < NT < MT. The carbon equivalent values increased with depth. The high ratio between C-WB and C-CS ($R2= 0.75$, $p= 0.0001$) justifies the use of correction factors.

Acknowledgements

To the Center for Nuclear Energy in Agriculture (Centro de Energia Nuclear na Agricultura) (CENA-USP) and to the Soil Organic Matter Laboratory (Laboratório de Matéria Orgânica do Solo) (Labmos - UEPG), for assistance in conducting the experiment.

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Table-1. ANOVA of the experiment

Causes of variation	TOC (WB*)	TOC (CS**)
Treatment (T)	*	*
Depth (P)	*	*
T x P	*	*
C.V.(%)	13.98	5.66

TOC= Total organic carbon;
* WB = Carbon Walkley Black;
** CS = Carbon Dry Combustion.

Table-2. Soil chemical properties and particle size distribution for each site for 0- to 20-cm layer†.

Fertility attributes							Particle size fractions		
pH‡	Al^{3+}	H + Al	Ca^{2+}	Mg^{2+}	K+	P	Sand	Silt	Clay
CaCl2	cmolc dm^{-3}					g dm^{-3}	g kg^{-1}		
5.41	0.03	6.29	4.22	2.26	0.36	9.60	344	168	488

† Mean of six replicates.
‡ pH, 1:2.5 soil/$CaCl_2$ 1 M.

Table-3. Average between the total organic carbon (TOC) concentrations determined through Walkey Black and through dry combustion of an Rhodic Hapludox in different management systems (1).

Treatment	Depth, cm	TOC -WB ($g.kg^{-1}$)	TOC –CS ($g.kg^{-1}$)	Standard deviation		Correction Factor***
				WB*	CS**	
CT	0-2.5	34.33 Aa	36.22 Aa	6.66	2.76	1.11
	2.5-5	33.00 Ba	35.25 Aa	3.00	3.04	1.08
	5.0-10.0	31.34 Ba	33.71 Aab	1.53	1.65	1.07
	10.0-20.0	27.67 Aab	29.99 Ab	5.86	1.45	1.03
	20.0-40.0	22.00 Ab	23.93 Ac	2.00	2.98	1.14
Average		**29.67 B**	**31.82 A**	**3.81**	**2.37**	**1.09**
MT	0-2.5	37.00 Aa	38.16 Aa	1.00	0.04	1.03
	2.5-5	34.67 Ba	37.15 Aa	3.06	0.29	1.19
	5.0-10.0	27.67 Bab	36.23 Aa	4.16	1.57	1.11
	10.0-20.0	23.67 Bb	30.58 Ab	2.52	1.51	1.10
	20.0-40.0	23.67 Ab	23.26 Ac	11.55	0.38	1.34
Average		**29.34 B**	**33.07 A**	**4.46**	**0.76**	**1.18**
NTC	0-2.5	44.50 Aa	40.01 Aa	7.51	2.75	1.08
	2.5-5	40.00 Aab	38.85 Aa	3.46	1.29	1.05
	5.0-10.0	36.50 Aabc	37.38 Aa	2.65	2.31	1.06
	10.0-20.0	28.00 Abc	28.90 Ab	2.00	1.33	1.02
	20.0-40.0	25.50 Ac	25.16 Ab	4.04	1.20	1.08
Average		**34.90 A**	**34.06 A**	**3.93**	**1.78**	**1.06**
NT	0-2.5	46.00 Ba	49.11 Aa	1.15	0.64	1.04
	2.5-5	37.25 Aab	40.72 Ab	1.53	2.79	1.07
	5.0-10.0	30.50 Abc	32.88 Ac	1.73	1.59	1.10
	10.0-20.0	25.50 Bcd	28.17 Ad	0.58	1.43	1.09
	20.0-40.0	19.75 Bd	24.07 Ae	0.58	0.50	1.25
Average		**31.80 B**	**34.99 A**	**1.11**	**1.39**	**1.11**

(1)Averages with equal small letters in columns (comparison between layers within each treatment) and capital letters in the lines (comparison between methods - WB x CS) did not differ among themselves by the Tukey test at 5% probability. TOC= Total organic carbon; * WB = Walkley Black; ** CS = Dry Combustion; *** Correction Factor (%) = 100/ (C-WB*100/C-CS).
CT= Conventional Tillage; MT= Minimum Tillage; NTC= No-till with chisel plowing and NT= No till.

Table-4. Averages of C concentrations at different depths

	C Concentration ($g.kg^{-1}$)		Standard Deviation	
Depth (cm)	**TOC (WB*)**	**TOC (CS**)**	**TOC (WB*)**	**TOC (CS**)**
0- 2.5	39.2	41.1	6.59	6.56
2.5- 5.0	35.8	38.1	3.57	2.86
5.0- 10.0	31.0	34.7	3.62	2.41
10.0- 20.0	26.0	29.4	3.28	1.60
20.0- 40.0	21.6	25.1	3.98	3.81

TOC= Total organic carbon;
* WB = Walkley Black;
** CS = Dry Combustion.

Figure-1. Ratio between carbon determined through Walkey Black (C-WB) and the carbon determined through dry combustion (C-CS) of an Rhodic Hapludox in different management systems. (a) Conventional Tillage, (b) Minimum Tillage, (c) No-till with chisel plowing and (d) No till

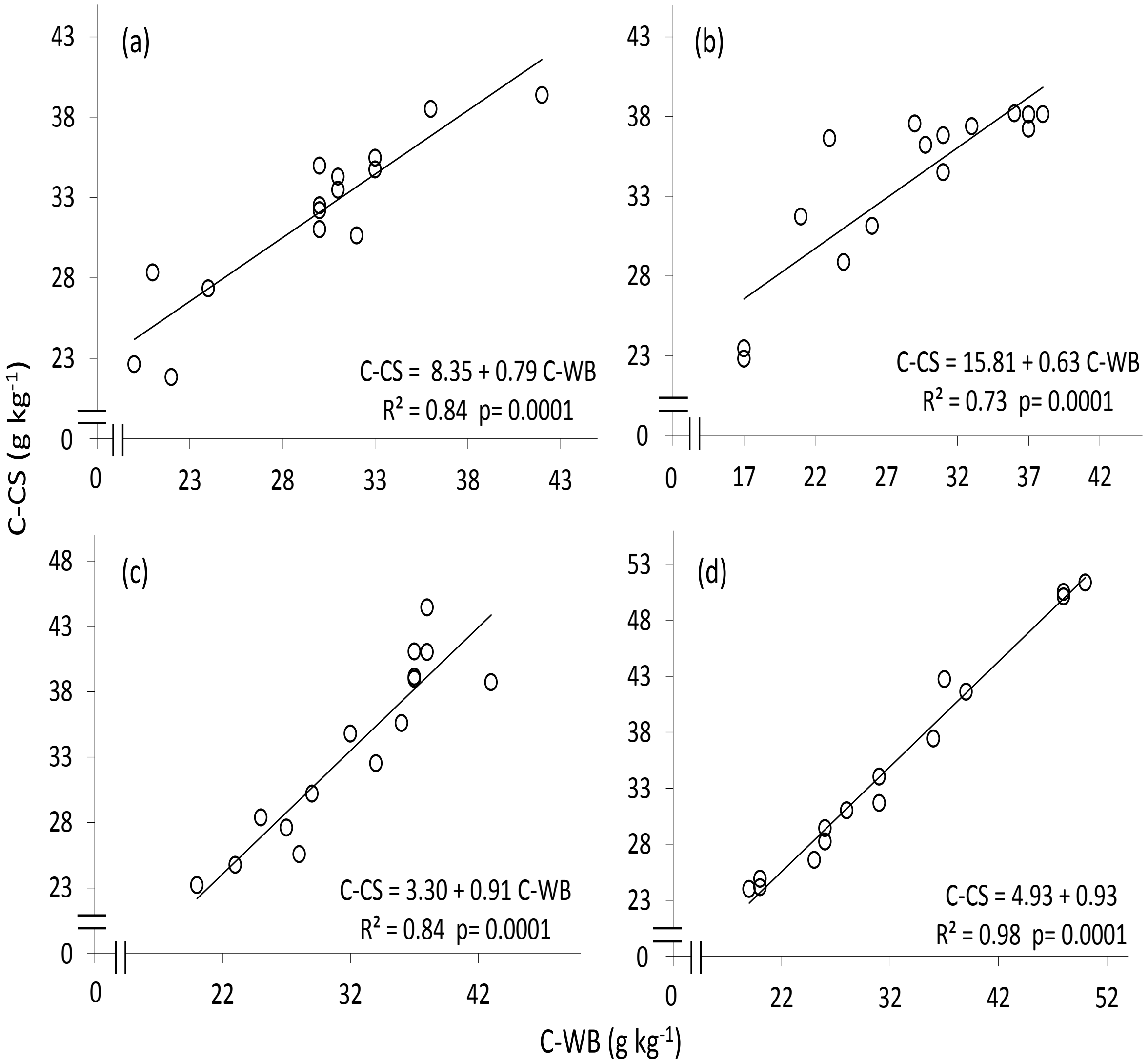

Figure 2. Ratio between the carbon determined through Walkey Black (C-WB) and the carbon determined through dry combustion (C-CS) of an Rhodic Hapludox at different soil depths. (a) 0- 2.5, (b) 2.5- 5.0, (c) 5.0- 10.0, (d) 10- 20 and (e) 20- 40 cm

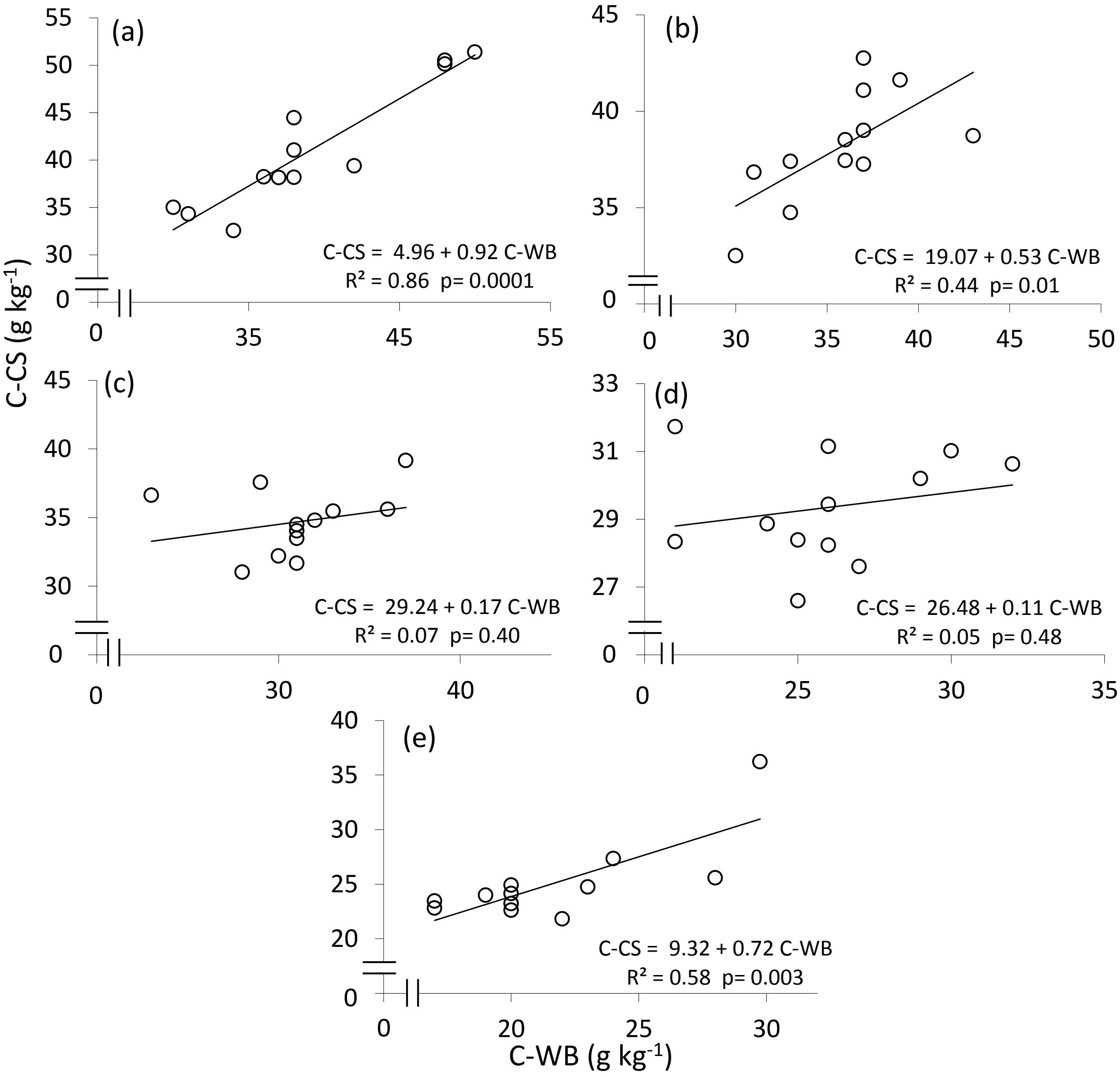

Figure 3. Ratio between the carbon determined through Walkey Black (C-WB) and the carbon determined through dry combustion (C-CS) of an Rhodic Hapludox.