Stratification Ratio as Soil Carbon Sequestration Indicator in Oxisol and Alfisol Under No-Tillage

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1. Introduction

Soil organic carbon (SOC) stocks generally decrease from topsoil to subsoil layers. This is due to continuous aboveground carbon input by plant and animal residues and the absence of soil disturbance in natural ecosystems [1]. A similar phenomenon occurs in long-term no-tillage systems, promoting stratification of SOC stocks between topsoil and the adjacent soil layers [2,3,4].

The topsoil layer mediates energy, water, nutrient and gas exchanges between soil and atmosphere and therefore has vital importance for the functioning of ecosystems [2,3]. Most of these processes are regulated by soil organic matter. NT systems decrease SOC losses due to biological oxidation or soil erosion [5]. Continuous deposition of vegetal and animal residues on the soil surface and minimum soil disturbance under NT enhances SOC stabilization mechanisms, allowing increase of SOC stocks [3,4,6]. SOC accumulation in topsoil layers provides higher resistance to erosion and compaction [7], increases water infiltration and enhances aggregate and pore stability [2,8,9,10]. Thus, increase of SOC stocks, and consequently increase of the carbon stratification ratio (CSR), in the topsoil can be a good indicator of soil management quality [3,4,11].

CSR is the ratio between SOC stocks from two soil layers, usually the topsoil, with strong influence of soil and crop management practices, and the adjacent soil layer, which is less affected by these operations [2]. CSR values of 3.4, 2.0, and 2.1 were observed in NT soils from Georgia and Texas (USA) and Alberta (Canada), respectively [2]. The higher CSR values were related with agro-ecosystems which provided the maintenance or increase of soil

quality. Additionally, CSR values higher than 2.0 would be unexpected in degraded soils from temperate climate regions.

CT managed soils from Alabama, Georgia, South and North Carolinas and Virginia in the USA had an average CSR of 1.4 [12]. CSR increased to 2.8 after ten years of NT adoption on the average of the evaluated soils. Three different soils from Virginia with original CSR values of 1.5 under CT, showed CSR of 3.6 after 14 years of NT adoption. Another study verified that CSR in a soil from the southeastern USA increased from 2.4 to 3.1 five years after conversion from CT to NT, reaching a CSR of 3.6 twelve years after the conversion [11].

Tropical NT soils have CSR values generally lower than temperate NT soils. CSR values of 1.48 (450 g clay kg⁻¹ soil) and 1.73 (600 g clay kg⁻¹ soil) were reported in Oxisols under NT from Southern Brazil [3,13]. These CSR values (SOC stock ratio of the 0-5 and 5-10 cm soil layers) had a close relationship with soil carbon sequestration rates verified in the 0-10 cm soil layer in these soils. Therefore, CSR could be used as an indicator of carbon sequestration in NT managed soils when historical data is not available for calculation of carbon sequestration rates. Furthermore, this procedure can be a tool to contribute to carbon stock inventories.

2. Material and methods

2.1. Description of the experimental areas

This study was carried out using two long-term experiments in southern Brazil. The first one was established in 1985 in Cruz Alta, state of Rio Grande do Sul, Brazil (28°33'S 53°40'W, altitude of 409 m). The local climate is subtropical humid (Cfa 2a according to the Köppen classification) with mean annual rainfall and temperature of 1,774 mm and 19.2 °C, respectively. The highest mean temperatures (30.0 °C) are registered in January and the lowest (8.6 °C) are registered in June [14]. The soil is a dystroferic Hapludox (referred to in the text as Oxisol) with 4.7% slope and predominance of kaolinite and iron oxides (63.5 g kg⁻¹) (Campos et al., 2011). The second experiment was established in 1991 in Santa Maria, state of Rio Grande do Sul, Brazil (29°43'S 53°42'W, altitude of 86 m). The local climate is subtropical (Cfa in the Köppen classification) with mean annual rainfall and temperature of 1,769 mm and 19.3 °C, respectively [14]. The soil is a dystrophic Paludalf (referred to in the text as Alfisol) with 5.5% slope, a moderate A horizon and clay loam texture. Further soil characteristics are presented in Table 1.

The long-term experiment in the Oxisol site had a split plot design with two soil tillage systems as the main plots (i.e., conventional tillage (CT) and no-tillage (NT)) and three crop systems in the subplots without replications: a) succession R₀ - soybean (*Glycine max* L Merrill)/wheat (*Triticum aestivum* L.); b) winter rotation R₁ - wheat/soybean/oat (*Avena strigosa* Schreber)/soybean; c) summer and winter rotation R₂- wheat/soybean/oat/soybean/oat+vetch (*Vicia sativa* L. Walp)/maize (*Zea mays* L.)/forage radish (*Raphanus sativus* var. *oleiferus* Metzger.). The

City	Soil	Layer	Carbon content	pH H ₂ O	Phosphorus	Potassium	Sand	Silt	Clay
		m	g kg ⁻¹		mg dm ⁻³		g kg ⁻¹		
Cruz Alta, RS, Brazil	Oxisol	0-0.20	19.0	4.5	19	82	310	120	570
Santa Maria, RS, Brazil	Alfisol	0-0.20	14.2	4.5	1.8	33	660	253	87

⁽¹⁾ Source: Adapted from [15,16,17].

Table 1. Main soil characteristics at the time of establishment of the experiments.

soil was amended with 5 Mg ha⁻¹ of lime at the time of establishing the experiment in 1985. Wheat and maize received 60 and 90 kg N ha⁻¹, respectively. R₁ and R₂ crop systems received 52 and 62 kg P₂O₅ ha⁻¹ yr⁻¹ and 75 and 105 kg K₂O ha⁻¹ yr⁻¹, respectively, in the first 15 years of the experiment [18]. Afterwards, phosphorus and potassium amendment was standardized at 50 kg P₂O₅ and K₂O ha⁻¹ yr⁻¹. Further experimental details are available in [19].

The long-term experiment in the Alfisol site had a completely randomized block design with six treatments and two replications: a) maize + jack beans (*Canavalia ensiformis* DC)/soybean (M/JB); b) bare soil (BS); c) maize/fallow/soybean(M/F); d) maize/ryegrass (*Lolium multiflorum* Lam.) + vetch/soybean (M/R); e) maize + velvet beans (*Stizolobium cinereum* Piper & Tracy)/soybean (M/VB); and f) maize/forage radish/soybean (M/FR). Details regarding the experiment are described in [17,20]. Soil acidity was corrected by the application of 3.5 Mg ha⁻¹ of lime in 1991 and 2 Mg ha⁻¹ of lime in 1996. The nitrogen fertilization rate for maize was 130 kg N ha⁻¹ in the M/F and M/R treatments and 65 kg N ha⁻¹ in the M/VB treatment. Further experimental details are described in [17]. The average carbon input values through plant residue in both long-term experiments are presented in Table 2.

2.2. Soil sampling and determination of SOC content

Soil samples were collected in 2001 (T₁) and 2007 (T₂) in the Alfisol site and in 2004 (T₁) and 2007 (T₂) in the Oxisol site. Small trenches (0.3x0.3x0.15 m, LxWxD) were opened in the plots and samples were collected with a spatula. The thickness of the sampled soil layers (0-0.05 and 0.05-0.15 m) was based on [10,11], considering that SOC stock increment due to changes in soil and crop management practices occurs mainly in the topsoil layer (0-0.05 m). Soil bulk density was determined in undisturbed soil samples collected from the same layers, by using steel rings measuring 0.05x0.04 m (diameter x height) [21]. Soil samples used for C/N analysis were air dried and root and plant residues were manually removed. Then the samples were finely ground in a porcelain mortar. SOC content was determined in soil samples collected at T₁ (2001 and 2004 in the Alfisol and Oxisol sites, respectively) by humid combustion [22,23], and by dry combustion using an elemental C/N analyzer (Flash EA 1112 Series ThermoFinnigan) for samples collected at T₂ (2007 for both sites). The SOC stocks were calculated based in equivalent

Soil	Tillage systems	Management	Carbon input
			Mg ha ⁻¹
Oxisol	CT	R ₀ ¹	3.54
	CT	R ₁ ²	4.48
	CT	R ₂ ³	5.04
	NT	R ₀	3.90
	NT	R ₁	4.82
	NT	R ₂	6.00
Alfisol	NT	M/JB ⁴	4.07
	-	BS ⁵	0.00
	NT	M/F ⁶	1.88
	NT	M/R ⁷	3.76
	NT	M/VB ⁸	4.51
	NT	M/FR ⁹	4.10

¹R₀ = succession soybean/wheat;
²R₁ = wheat/soybean/oat/soybean;
³R₂ = wheat/soybean/oat/soybean/oat+ vetch/maize/radish;
⁴M/JB= maize + Jack bean/soybean;
⁵BS= bare soil;
⁶M/F= maize/fallow/soybean;
⁷M/R= maize/ryegrass+ vetch/soybean;
⁸M/VB= maize/velvet bean; ⁹M/FR= maize/forage radish/soybean.

Table 2. Mean annual carbon input through crop residues in two experimental areas.

soil masses [24], taking as references the treatments M/F and CT R₀ (T₁) for the Alfisol and Oxisol sites, respectively.

2.3. Calculation of carbon stratification ratio (CSR)

The CSR was calculated as proposed by [2], by the ratio of SOC stocks of the superficial soil layer (0- 0.05 m) and SOC stocks of the adjacent soil layer (0.05- 0.15 m) in a given treatment. The temporal variation in CSR (Δ CSR) was obtained by the difference of CSR determined in the second period (T₂) and the CSR in the first (T₁). Soil carbon sequestration rate was deter-

mined by the temporal variation of SOC stocks in each treatment in the same period (six and three years for the Alfisol and Oxisol sites, respectively).

2.4. Statistical analysis

The results were submitted to analysis of variance (ANOVA) using the software SISVAR 5.0 [25] and the means were compared by the Tukey test ($p < 0.05$). The regression analysis was performed through the software JMP IN version 3.2.1 [26], using the F test ($p < 0.05$).

3. Results and discussion

3.1. Carbon input and CSR in Oxisol and Alfisol

Significant linear relationships between carbon input and CSR were observed for both the Oxisol ($p = 0.003$; Figure 1a) and Alfisol ($p = 0.0004$; Figure 1b) sites. These results indicate that increase of carbon input leads to direct increase of CSR, confirming the strong influence of aboveground carbon input for SOC accumulation in the topsoil layers for both soil types.

The average CSR of the Alfisol was 1.66 ± 0.33 (Table 4), which was 21.1% higher than the CSR of the Oxisol (1.31 ± 0.25) (Table 5). The CSRs of these subtropical climate soils were lower than previously reported for temperate soils, where a NT soil from the southeastern U.S. achieved CSR of 3.6 after 12 years [11]. Higher CSR values in temperate climate soils could be related to the less C-oxidative environment in relation to subtropical or tropical climate soils. However, the CSR values observed in our study were close to the results from other Brazilian tropical soils [4,13]. A CSR value of 2.0 was suggested as a lower limit for soil quality in temperate climate regions [2], yet a CSR value of 1.5 would be more appropriate for subtropical or tropical soils [3,4,27]. Another study proposed that declining quality NT soils would result in $CSR < 1.0$, soils in transition from CT to NT would have CSR between 1.0 and 1.25, a consolidated NT soil would have CSR values ranging from 1.25 to 1.5, and a rising/high quality NT soil would achieve $CSR > 2.0$ [27]. Comparing our results with that proposed classification, treatments NT R₁ and NT R₂ in the Oxisol, and M/JB, M/VB, M/FR and M/R in the Alfisol, would be considered rising/high quality systems. However, treatments NT R₀ in the Oxisol and M/F in the Alfisol would be classified as consolidated systems, while treatments CT R₀, CT R₁ and CT R₂ in the Oxisol and BS in the Alfisol would be classified as systems under transition.

3.2. CSR and carbon sequestration rates

The relationship between CSR and C stocks ($Mg\ ha^{-1}$) was linear and significant for both the Oxisol ($p = 0.0001$; Figure 2a) and Alfisol ($p = 0.0001$; Figure 2b). Similar results were observed in others Oxisols from Brazil [4,28].

The linear and significant relationships observed between ΔCSR (0-0.05: 0.05-0.15 m) and ΔC stock (0-0.05m layer) in the Oxisol ($p = 0.0001$; Figure 3a) and Alfisol ($p = 0.0001$; Figure

Figure 1. Relationship between annual carbon input and CSR (0-0.05: 0.05-0.15 m) under no tillage. (a) Oxisol. (b) Alfisol. CSR = carbon stratification ratio.

3b), suggest that C sequestration rates are associated with increasing SOC stratification in the soil profile [3,4,28]. Those studies related this effect with the aboveground carbon input by crop residues which are maintained on the soil surface and also with the higher SOC physical protection inside soil macroaggregates under NT. The slope of the adjusted linear equation for the Oxisol was almost twice that for the Alfisol. These results could be associated with the higher clay and Fe and Al oxides content in the Oxisol, favoring C stabilization [29]. The importance of the organo-mineral interaction for C stabilization in Oxisols has been previously reported in the literature [30,31,32]. Also, the linear relationship observed between variables indicates that both soils continue accumulating SOC.

Nevertheless, after more and 20 years, no evidence of SOC saturation was found in any of the evaluated treatments in both soils [33,34].

Both CT and NT treatments had linear relationship between Δ CSR and Δ SOC in the Oxisol site. This significant relationship ($p=0.006$) indicates that high carbon input (R_2) could promote SOC accumulation even under CT (Figure 4a), while low carbon inputs leads to depletion of SOC stocks under intensive soil disturbance (R_0 and R_1). This relationship was more pronounced under NT ($p=0.0005$) with higher C sequestration rates (Figure 4b). These results confirm the soil as an atmospheric CO_2 sink when conservation agriculture (NT) is associated with high carbon inputs through crop residues [16,35,36,37].

Figure 2. Relationship between CSR (0-0.05: 0.05-0.15 m) and SOC stocks in the 0-0.05 m layer. (a) Oxisol. $T_1=2004$; $T_2=2007$ (interval of 3 years). (b) Alfisol. $T_1=2001$; $T_2=2007$ (interval of 6 years) CSR = stratification relation.

Figure 3. Relationship between the temporal CSR variation ($\Delta \text{CSR} = T_2 - T_1$) and temporal SOC stock variation (ΔSOC stock = $T_2 - T_1$) in the 0-0.05 m layer. (a) Oxisol. T_1 =2004; T_2 = 2007 (interval of 3 years). (b) Alfisol T_1 =2001; T_2 = 2007 (interval of 6 years). CSR = stratification relation.

Figure 4. Relationship between the temporal CSR variation ($\Delta CSR = T_2 - T_1$) and temporal SOC stocks variation (ΔSOC stock = $T_2 - T_1$) in the 0-0.05 m layer of the Oxisol. (a) conventional tillage, (b) no tillage. CSR = stratification relation.; T_1 = 2004; T_2 =2007 (interval of 3 years).

4. Conclusions

The linear relationship between ΔCSR and ΔSOC in the topsoil layer indicates that SOC accumulation is related to carbon stratification in the soil profile. This relationship was more pronounced in the Oxisol than in the Alfisol. Higher CSR values were observed with the association of NT and intensive crop rotation (NT R_2) in the Oxisol. Use of cover crops promoting high carbon and nitrogen input in the soils led to higher CSR values in the Alfisol. Although the CSR values observed in subtropical soils (our study) were lower than those

reported for temperate climate soils, this index was efficient for evaluation of carbon sequestration in agricultural soils.

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