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Scientific Article

CO_2 emissions and chemical properties of Histosols under drainage and liming in a controlled environment

Emissões de co_2 e propriedades químicas de Organossolos sob drenagem e calagem em ambiente controlado

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ABSTRACT - Climate change integrates global warming, the water crisis and socio-environmental impacts. Carbon sequestration in the soil is a strategy to mitigate these effects, and Histosols (*Organossolos*) are important due to their high C stocks. However, they have limitations such as high acidity and low oxygenation, requiring liming and drainage. However, if carried out inappropriately, these operations cause degradation of soil organic matter (SOM) and greenhouse gas (GHG) emissions. The aim was to investigate the effects of combining these practices on fertility, C content, humic fractions and C-CO_2 emissions in *Organossolo Tiomórfico* from southeastern Brazil. The trial was conducted in a 2 × 3 factorial design (with and without liming × drainage levels: 30, 60 and 90% of field capacity), with 4 replications. The samples were evaluated at two different times (30 days of incubation and another 30 days of incubation). CO_2 release was quantified at regular intervals and C-CO_2 losses were estimated in Mg ha^{-1}. In each phase, soil chemical attributes and SOM indices were analyzed. The practices adopted altered the carbon content of humic substances, microbial respiration and soil fertility. Fulvic acid contents decreased under lower drainage, and humic acid decreased with $CaCO_3$ application and waterlogging. Liming increased C-CO_2 emissions, regardless of drainage. C-CO_2 losses were ± 4.1 Mg ha^{-1} in the first phase and ± 1.6 Mg ha^{-1} in the second phase of the experiment. It can be concluded that liming, in the short and medium term, increases C-CO_2 losses in Histosols.

Keywords: Climate change. Greenhouse gases. Soil organic matter.

RESUMO - Mudanças climáticas integram aquecimento global, crise hídrica e impactos socioambientais. O sequestro de carbono no solo é uma estratégia para mitigar esses efeitos, sendo Organossolos importantes pelos elevados estoques de C. Porém, apresentam limitações como elevada acidez e baixa oxigenação, exigindo calagem e drenagem. Contudo, se realizadas inadequadamente, promovem degradação da matéria orgânica do solo (MOS) e emissões de gases de efeito estufa. Objetivou-se investigar os efeitos da combinação dessas práticas na fertilidade, teores de C, frações húmicas e emissões de C-CO_2 em Organossolo Tiomórfico do sudeste do Brasil. O ensaio foi conduzido em DIC, em esquema fatorial 2 × 3 (com e sem calagem × níveis de drenagem: 30, 60 e 90% da capacidade de campo), com 4 repetições. As amostras foram avaliadas em dois momentos (30 dias de incubação; e nova incubação por mais 30 dias). Quantificou-se a liberação de CO_2 em intervalos regulares, estimando-se perdas de C-CO_2 em Mg ha^{-1}. Em cada fase, analisaram-se atributos químicos do solo e índices da MOS. As práticas adotadas alteraram teores de carbono das substâncias húmicas, a respiração microbiana e a fertilidade do solo. Houve redução nos teores de ácido fúlvico com menor drenagem e de ácido húmico com $CaCO_3$ e lâmina d'água. A calagem aumentou as emissões de C-CO_2, independentemente da drenagem. As perdas de C-CO_2 foram de ± 4,1 Mg ha^{-1} na 1ª fase e ± 1,6 Mg ha^{-1} na 2ª do experimento. Conclui-se que a calagem, a curto e médio prazo, eleva as perdas de C-CO_2 em Organossolo.

Palavras-chave: Mudanças climáticas. Gases de efeito estufa. Matéria orgânica do solo.

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INTRODUCTION

Climate change is one of the main challenges of the twenty-first century (WHITE, 2024). Despite being linked to global warming, it integrates variations in the water cycle, generating intense droughts and recurrent floods, affects ecosystems and causes socioeconomic impacts (UNDP, 2023).

Human impact has become a highlight in climate change, driven by greenhouse gas (GHG) emissions from industrial and agricultural activities, and changes in land use forms (WANG, 2023). Agricultural activities and conversion of native areas into agrosystems account for 13% of global C dioxide (CO_2) emissions, with Brazil being responsible for 4.4% of these emissions (AZEVEDO; SARTORI; CAMPOS, 2018).

Therefore, the country has aligned its policies with the Sustainable Development Goals (SDGs) and the Paris Agreement, aiming to neutralize emissions by 2050. Carbon (C) sequestration in agricultural soils emerges as a solution in this scenario (FRONZA et al., 2024), especially in *Organossolos* (Histosols), and this class is responsible for storing approximately 4700 tons of C per hectare of soil (TEMMINK et al., 2022). However, inadequate management practices lead to the loss of these reserves.

Due to oxygen deficiency and high contents of soil organic matter (SOM), which gives *Organossolos* high levels of aluminum (Al^{3+}) and low pH values, drainage and liming are the main management practices in these soils (SANTOS et al., 2024; OLIVEIRA FILHO et al., 2021). These practices, if carried out inappropriately in *Organossolos*, cause the emission of CO_2 and N_2O into the atmosphere due to the increase in the oxidation and decomposition rates of SOM, as these emissions last for decades after drainage, given the large amounts of SOM available (CONCHEDDA; TUBIELLO, 2020; JIA; LI; FENG, 2024). In this context, the aim is to identify sustainable management practices for *Organossolos*, especially in cases of subsidence caused by drainage or excessive use of limestone.

FAO recommended indicators, such as organic C and biological activity, for the evaluation of sustainable soil management, considering their efficiency, practicality and cost-benefits. Soil organic C reflects the chemical, physical and biological state of the soil, responding to changes through the implementation of more sustainable practices. Biological activity, on the other hand, can be measured by soil microbial respiration, an efficient indicator in monitoring the impacts of agricultural management (CROOKSTON et al., 2023).

To understand the effects related to the practices employed in these soils, we raised the following questions: (1) can variations be observed in soil attributes when liming is carried out in association with drainage in *Organossolos*, since these factors accelerate the decomposition of SOM?; (2) can these variations be monitored by the evaluation of humified SOM and soil microbial respiration, hence allowing the estimation of C-CO_2 losses?; (3) can the residual effect of liming plus drainage contribute to the changes in these attributes?; and (4) is it possible to suggest sustainable management practices of these *Organossolos*, contributing to the mitigation of GHG emissions? To answer these questions, the study aimed to investigate the effect of liming and drainage levels on soil fertility, C contents, humic fractions, and C-CO_2 emission in *Organossolo Tiomórfico* samples from the southeastern region of Brazil.

MATERIAL AND METHODS

The study was carried out from April to June 2023, with samples collected in the municipality of Rio de Janeiro (RJ), southeastern Brazil (Figure 1). The climate in the region is classified as Aw (dry winter and rainy summer), with average annual rainfall of approximately 1300 mm and average annual temperature between 23.6 and 24.7 ºC (FIALHO; MACHADO, 2024).

The soils in the study area were classified as *Organossolos Tiomórficos* (Histosols) (SANTOS et al. 2018) and have poor drainage conditions, high levels of SOM and sulfur (S), and conventional soil management carried out for 80 years for vegetable production.

In 2020, after heavy rains in the state of Rio de Janeiro, the area stopped being cultivated due to the inefficiency of the drainage process. At the time of collection, natural regeneration was observed in the area, with a predominance of species of the *Gramineae* family.

Three random points, 15 m apart from each other, were selected in this area. At each point, soil pits of up to 40 cm were opened and 4 undisturbed samples were collected with the aid of a Kopeck ring in order to determine bulk density; disturbed soil material of the organic horizon (H) was also collected. The samples were packed in bags and transferred to a drying shed where the disturbed material was homogenized with the aid of a shovel and a concrete mixer. The undisturbed samples were taken to the oven at 105 ºC and kept until complete drying (constant weight) to determine soil bulk density.

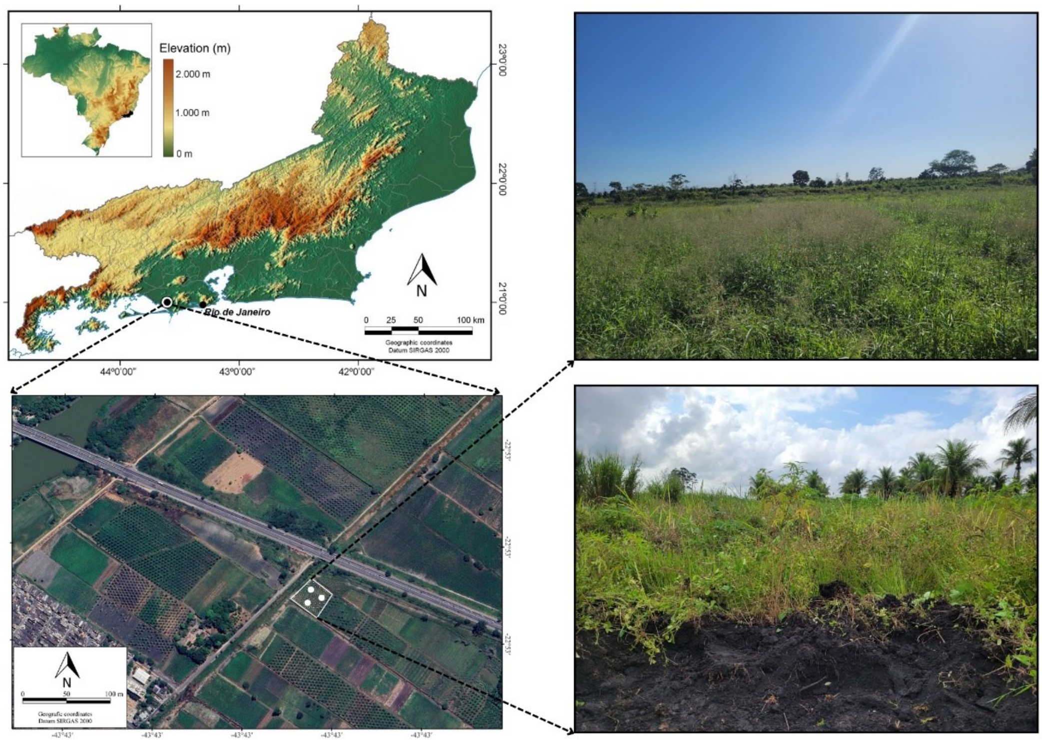


Figure 1. Location map of the area where the soil samples were collected. Rio de Janeiro, RJ, Brazil (22º53’25.26” S and 43º42’40.32” W).

After homogenization of the disturbed material, samples were randomly collected, air-dried, pounded to break up clods and passed through a 2.0-mm-mesh sieve to obtain the air-dried fine earth (ADFE) fraction, used for the evaluation of the soil field capacity and chemical characterization of the material, which was later used to carry out the laboratory test.

Field capacity was determined using the funnel method (LOSS et al., 2013). For chemical characterization of the material (Table 1), analyses were carried out to evaluate soil fertility, such as pH in water, Ca^{2+}, Mg^{2+}, Al^{3+}, Na^{+}, K^{+}, potential acidity (H+Al) and available P (Mehlich-1) (TEIXEIRA et al., 2017). Total organic C (TOC) was determined via wet oxidation of SOM according to Yeomans and Bremner (1988).

Humic substances (HS) were obtained by extraction and separation by means of the differential solubility of SOM in basic or acidic medium (fulvic and humic acids) and the residue (humin) (MENDONÇA; MATOS, 2005). Organic C in each of the humic fractions, fulvic acids (FA), humic acids (HA) and humin (HUM), was also determined according to Yeomans and Bremner (1988). The E4/E6 ratio was quantified according to Kumada (1987). After the analyses, the material was characterized with the following chemical, physical and organic matter characteristics, from 0 to 40 cm (Table 1).

Table 1. Characterization of the chemical attributes, bulk density and soil organic matter fractions of the *Organossolo Tiomórfico* used in the test.

Sample	Sorption complex							
	pH (H_2O)	Ca^{2+}	Mg^{2+}	Al^{3+}	Na^{+}	K^{+}	H+Al	P
		---------- $cmol_c\ dm^{-3}$ ----------						$mg\ dm^{-3}$
1	3.69	0.9	0.6	4.5	0.01	0.01	14.8	51.0
2	3.73	0.9	0.5	4.5	0.01	0.01	13.7	48.0
3	3.51	0.9	0.5	4.5	0.01	0.01	14.5	52.0
	Organic carbon					Bulk density		
	FA	HA	HUM	TOC	E4/E6	$Mg\ m^{-3}$		
	---------- $g\ kg^{-1}$ ----------							
1	33.6	36.7	47.5	93.3	1.6	0.67		
2	43.7	33.1	43.6	90.8	1.2	0.75		
3	42.8	35.6	48.3	103.0	1.4	0.66		

pH: Active acidity; Ca^{2+}: Exchangeable calcium; Mg^{2+}: Exchangeable magnesium; Al^{3+}: Exchangeable aluminum; Na^{+}: Exchangeable sodium; K^{+}: Exchangeable potassium. H+Al: Potential acidity; P: Available phosphorus; FA: Carbon of the fulvic acid fraction; HA: Carbon of the humic acid fraction; and HUM: Carbon of the humin fraction; and TOC: Total organic carbon.

After chemical characterization and characterization of the field capacity of the material, the evolution of C-CO_2 began to be evaluated in the laboratory at 25 ºC (by means of refrigeration) according to the method developed by Mendonça and Matos (2005).

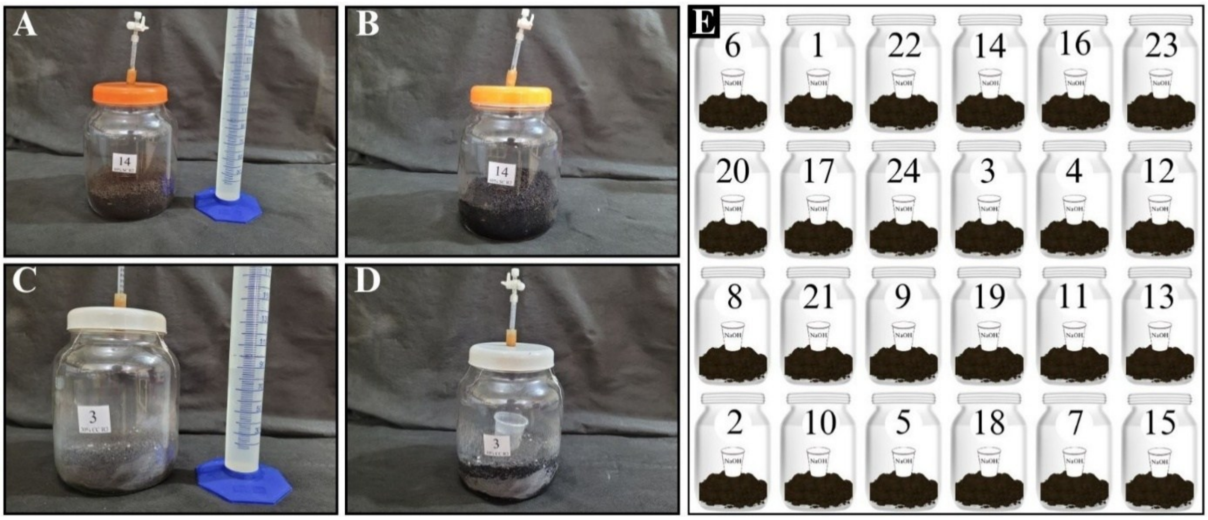
The experiment was designed in a completely randomized design (CRD), in a factorial scheme, with 2 different liming doses (with and without liming), using $CaCO_3$ (A.R.) as the source, and 3 different water depths, simulating different drainage conditions (30%, 60% and 90% of the field capacity), totaling 6 treatments. Each treatment had 4 replicates, totaling 24 experimental units (glass vials). Liming was performed using the base saturation method (RAIJ et al., 1997). To this end, the soil base saturation was raised to 70%, a value considered adequate for most crops.

Thus, the following treatments were established, namely: WoL30: without liming and 30% of field capacity; WoL60: without liming and 60% field capacity; WoL90: without liming and 90% field capacity; WiL30: with liming and 30% field capacity; WiL60: with liming and 60% field capacity; and WiL90: with liming and 90% field capacity.

To evaluate the CO_2 released, a vial containing 30 mL of 0.5 mol L^{-1} NaOH solution to capture the C-CO_2 and another with 30 mL of water to maintain humidity were added to each container (Figure 2). Evaluations were carried out at different times: two at 24-hour intervals, followed by two at 48-hour intervals, and the others every 72 hours, totaling 30 days of monitoring. The daily release of C-CO_2 was expressed in mg of C-CO_2 500 g^{-1} soil, according to the intervals between measurements. The accumulation of C-CO_2 (C-CO_2-Total) was equal to the sum of the values obtained in each evaluation and converted into $g\ kg^{-1}$. CO_2 losses were also estimated through C-CO_2 emission in Megagram per hectare ($Mg\ ha^{-1}$).

At the end of the experiment, 100 g of the material was removed from each container, air-dried, pounded to break up clods and passed through a 2.0-mm-mesh sieve to obtain the ADFE again. In this material, the contents of TOC, FA and HA, the values of the E4/E6, FA/TOC, HA/TOC and HA/FA ratios, and the contents of fertility attributes were determined in order to compare the effects of liming and different water depths on soil chemical attributes.

In order to evaluate the residual effect of carbonate application, the material that remained in the glass containers was air-dried for 30 days, with manual turning every 3 days. After complete drying, the samples were moistened again, adding the amount of water necessary to reach the values of 30, 60 and 90% field capacity, and the evolution of C-CO_2 over 30 days was evaluated for another cycle. At the end of the second incubation, the same chemical analyses were repeated.



A: Treatment without liming before the addition of the water depth corresponding to 60% field capacity for drainage simulation; B: Treatment without liming after the addition of the water depth; C: Treatment with liming before the addition of the water depth corresponding to 30% field capacity for drainage simulation; D: Treatment with liming after the addition of the water depth; and E: Sketch representing the arrangement of samples on a bench in the laboratory.

Figure 2. Containers with hermetic seal containing samples of *Organossolo Tiomórfico* used to evaluate the evolution of C-CO_2 emission when subjected to liming and drainage simulation.

The data of TOC, FA, HA, FA/TOC, HA/TOC, HA/FA, E4/E6, C-CO_2-Total and C-CO_2 emission were tested for normality of the residuals by the Shapiro-Wilk test, and for the homoscedasticity of the variances by the Bartlett test. Variables that did not show normal distribution or homoscedasticity were transformed according to the Box-Cox test and were tested again. The data were evaluated by analysis of variance followed by Tukey test when the assumptions of normality and homoscedasticity were met (transformed or non-transformed variables). In cases in which the data transformation was inefficient, the Kruskal-Wallis test followed by Fisher's least significant difference criterion were used as a non-parametric analysis. All tests were performed at 5% significance level in R software, using the "ExpDes.pt" packages.

The effects of treatments and incubation periods on the variables analyzed were interpreted by multivariate principal component analysis (PCA). To perform the PCA, the data (fertility attributes, TOC, FA, HA, FA/TOC, HA/TOC, HA/FA, E4/E6, C-CO_2-Total and C-CO_2 emission) were initially standardized to zero mean and unit variance ($\mu = 0$, $\sigma = 1$). PCA was performed as a function of Pearson's correlation matrix, based on the evaluated attributes. The first two principal components (PC1 and PC2) were used, based on the variables with correlations of $-0.70 \geq r \geq 0.70$ (high correlation) with these axes of formation, which added up to a cumulative variance of 70% (PC1 + PC2). Multivariate analysis was carried out in R software using the "Ggplot2" package.

RESULTS AND DISCUSSION

Attributes of humified organic matter after first incubation

For TOC contents and values of the E4/E6 ratio, no significant effects of the evaluated factors were found (Table 2). In *Organossolos*, where SOM has a high degree of humification, most of the C is associated with more stable humic fractions, such as humin. The predominance of C in this fraction indicates greater stability and permanence of C in the soil (ARAÚJO et al., 2025), which contributes to avoiding variations in TOC contents and in the degree of C humification in the short term.

The effect of the interaction between the application of $CaCO_3$ (absence and presence of $CaCO_3$) and the control of water depth was observed mainly in the FA and HA fractions and in the FA/TOC and HA/FA indices in the first phase of the experiment (Table 2). For the FA fraction and FA/TOC ratio, the lowest results were found in the treatment that did not receive liming associated with a higher water depth (WoL90). This indicates that the lower drainage (higher water content in the soil) without the application of $CaCO_3$ disfavored the stabilization and accumulation of C in the most soluble, hydrophilic and acidic fraction of humic substances.

An inverse pattern was observed for the HA fraction and its contribution to TOC contents (HA/TOC), in which the highest values of these attributes were found in the treatments without liming and with lower levels of water depth (WoL30 and WoL60) (Table 2). These results indicate that liming resulted in a decrease in C contents, especially in the HA fraction. This fraction has a more complex molecular structure than the FA fraction, and it is possible to suggest that, due to the change in pH, increased microbial activity, and consequent increase in SOM mineralization, resulting from the application of $CaCO_3$, the transformation of these more complex molecules (HA) into simpler molecules (FA) has occurred (SANTOS et al., 2024). More complex, stable, and humified molecules of SOM, such as HA, act mainly on the C stock in soils, contributing to the sequestration of GHGs such as CO_2 and CH_4 (ARAÚJO et al., 2025).

Table 2. Organic matter attributes of *Organossolo Tiomórfico* after 30 days of incubation subjected to liming and different water depths.

Field Cap. (%)	TOC (g kg^{-1}) WoL	WiL	$\bar{X}_1$ (1)	Field Cap. (%)	FA (g kg^{-1}) WoL (2)	WiL (2)	$\bar{X}_1$
30	84.59	83.37	83.98 ns	30	4.79 aA	6.24 aA	5.56
60	85.14	83.86	84.50	60	4.12 aA	4.87 aA	4.46
90	83.79	87.30	85.54	90	2.57 bB	4.10 aA	3.33
$\bar{X}_2$ (1)	84.51 ns	84.84		$\bar{X}_2$	3.85	5.04	
CV%		2.70		CV%		38.30	
Field Cap. (%)	**HA (g kg^{-1}) WoL (3)**	**WiL (3)**	**$\bar{X}_1$**	**Field Cap. (%)**	**FA/TOC (%) WoL (2)**	**WiL (2)**	**$\bar{X}_1$**
30	19.15 aA	10.76 bA	14.96	30	5.70 aA	7.50 aA	6.60
60	16.83 aAB	9.26 bA	13.05	60	4.80 aA	5.70 aA	5.30
90	14.43 aB	9.21 bA	11.82	90	3.10 bB	4.70 aA	3.90
$\bar{X}_2$	16.80	9.75		$\bar{X}_2$	4.50	6.00	
CV%		11.00		CV%		39.40	
Field Cap. (%)	**HA/TOC (%) WoL**	**WiL**	**$\bar{X}_1$ (2)**	**Field Cap. (%)**	**HA/FA WoL (1)**	**WiL (1)**	**$\bar{X}_1$**
30	22.60	12.90	17.80 A	30	4.00 aB	2.00 bA	3.00
60	19.80	11.00	15.40 B	60	4.10 aB	2.10 bA	3.10
90	17.20	10.50	13.90 B	90	5.70 aA	2.30 bA	4.00
$\bar{X}_2$ (2)	19.90 a	11.50 b		$\bar{X}_2$	4.60	2.10	
CV%		11.10		CV%		18.10	
Field Cap. (%)	**E4/E6 WoL**	**WiL**	**$\bar{X}_1$ (1)**				
30	2.50	2.30	2.40 ns				
60	2.30	2.40	2.30				
90	2.20	2.50	2.30				
$\bar{X}_2$ (1)	2.30 ns	2.40					
CV%		10.90					

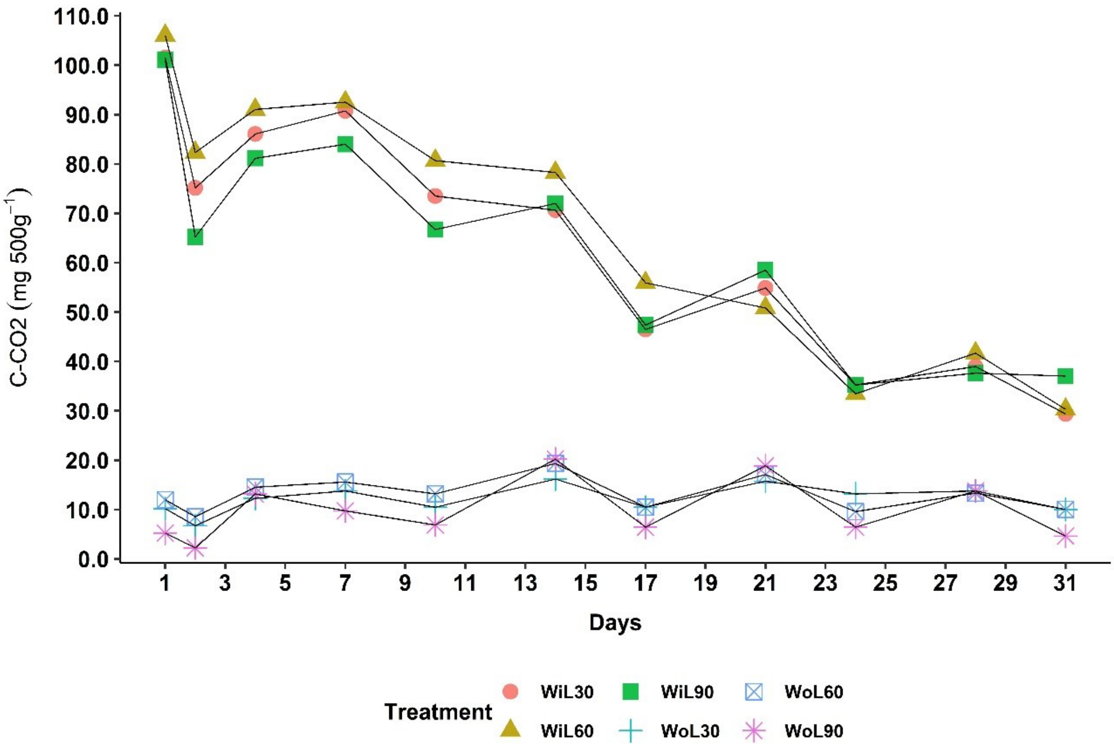
Means followed by the same lowercase letter in the row do not indicate differences in limestone application. Equal uppercase letter in the column does not indicate differences between the water depths. (ns)Absence of significance. $\bar{x}_1$: Overall mean of water depth; $\bar{x}_2$: Overall mean of limestone application. (1) ANOVA without data transformation + Tukey test ($p < 0.05$). (2) ANOVA with data transformation + Tukey test ($p < 0.05$). (3) Kruskal-Wallis test + Fisher's least significant difference for liming factor levels and Wilcoxon test for drainage factor levels ($p < 0.05$). WoL30: without liming and 30% field capacity; WoL60: without liming and 60%; WoL90: without liming and 90%; WiL30: with liming and 30%; WiL60: with liming and 60%; and WiL90: with liming and 90%. TOC: Total organic carbon; FA: Carbon of the fulvic acid fraction; FA/TOC: Percentage of carbon in the fulvic acid fraction relative to TOC; HA: Carbon of the humic acid fraction; HA/TOC: Percentage of carbon of the humic acid fraction relative to TOC; and HA/FA: Ratio of carbon of the humic acid and fulvic acid fractions; and E4/E6: E4/E6 ratio.

HA/FA ratio (soil carbon mobility) ranged from 2.00 in the WiL30 treatment to 5.70 in the WoL90 treatment. These results indicate the selective loss of the less labile fraction (HA) after the application of $CaCO_3$. Agricultural management practices that subject SOM to mineralization, such as drainage and liming, contribute to losses of C of the most soluble fractions of SOM, even in the short term. However, our results indicate that liming, at agricultural production levels, in *Organossolo* areas significantly affects less soluble fractions of SOM, such as HA, highlighting the potential of liming to favor the mineralization of stored C in the form of humic acid. In turn, the highest water depth affected the most soluble fraction (FA).

Evolution of C-CO_2 after the first incubation

Regarding the evolution of CO_2, it is observed that the application of $CaCO_3$ intensified the release of C in the form of CO_2 (Figure 3). In our study, we observed values of C-CO_2 lost in the treatments of WiL30, WiL60 and WiL90 ranging from 106.05 to 29.40 mg CO_2 500 g^{-1}, obtained, respectively, on the first and thirty-first days after liming. For the WoL30, WoL60 and WoL90 treatments, the values ranged from 19.35 to 2.25 mg CO_2 500 g^{-1}, obtained, respectively, on the fourteenth and second days after liming. It can be observed that the effects of liming associated with different amounts of water, simulating the effect of drainage depths, resulted in an increase in the rate of microbial respiration, generating C losses in the form of C-CO_2 up to 10 times greater than in samples of the treatments that did not receive liming (WoL), especially in the first 14 days of evaluation after $CaCO_3$ application (Figure 3).

When evaluating the differences in C-CO_2 losses between the treatments with and without liming, we observed the highest values in the WiL30, WiL60 and WiL90 treatments. However, if we evaluate the effect of the different water depths within each treatment (with and without liming), it is observed that for the liming treatment, the depth promoted an effect only on the tenth day, and the one corresponding to the lowest drainage (WiL90) had the lowest loss of C-CO_2 compared to the WiL30 and WiL60 treatments (Figure 3).



WoL30: without liming and 30% field capacity; WoL60: without liming and 60%; WoL90: without liming and 90%; WiL30: with liming and 30%; WiL60: with liming and 60%; and WiL90: with liming and 90%.

Figure 3. Evolution of C-CO_2 emission from *Organossolo Tiomórfico* subjected to different water depths and liming over 30 days.

$CaCO_3$ application to the soil modified the pH, raising the values from ± 4.0 to ± 6.0 (Table 3). This increase directly influences the biological dynamics of the soil, favoring the so-called priming effect, in which the increase in pH stimulates the microbial activity responsible for the decomposition of SOM, resulting in greater CO_2 release (MORAN-RODAS; JOERGENSEN; WACHENDORF, 2023). This increase in CO_2 emission occurs mainly due to the intensification of heterotrophic respiration, a process in which microorganisms decompose SOM and release CO_2 as a byproduct (ZHANG et al., 2022).

Liming, therefore, can play a significant role in the carbon balance in ecosystems under acidic soils. Sridhar et al. (2022) also observed this effect in forest soils, identifying short-term impacts of liming on the bacterial community. The authors found that 70% of bacterial taxa responded to liming in the short term, while only 30% showed long-term responses, which highlights that the effects of liming on the microbial community are immediate.

The values of C-CO_2 lost over the 30 days of evaluation (C-CO_2-Total) ranged from 0.22 to 1.49 g kg^{-1}, with the lowest values observed in the treatment without $CaCO_3$ associated with the lowest drainage (WoL90), while the highest values were observed in the treatment with liming and depth corresponding to 60% field capacity (WiL60) (Table 4). When we evaluated the liming factor, for all levels of drainage simulation tested, the values of C-CO_2-Total were higher in the treatments with liming. The effect of drainage was not significant, except when comparing the treatment without liming, in which the observed values were significantly lower at the depth corresponding to 90% field capacity (WoL90).

Table 3. Chemical attributes of *Organossolo Tiomórfico* after 30 days of incubation subjected to liming and different water depths and 30 days after incubation subjected to the residual effect of liming and different water depths.

Treatment	pH	Ca	Mg	Al	Na	H+Al	K	P
	H_2O			$cmol_c\ dm^{-3}$				$mg\ dm^{-3}$
1WiL30	6.21	19.09	1.32	0.00	0.02	7.45	1.96	17.66
2WiL30	6.48	20.98	0.98	0.00	0.03	5.78	3.42	3.70
1WoL30	3.87	0.95	1.08	6.12	0.02	20.40	2.74	14.22
2WoL30	4.17	1.15	1.08	4.53	0.02	22.03	3.47	3.60
1WiL60	6.16	20.90	3.63	0.00	0.02	7.80	2.74	15.53
2WiL60	6.25	21.18	1.00	0.00	0.02	5.40	3.09	3.57
1WoL60	4.82	1.04	1.24	4.66	0.02	23.83	3.92	17.46
2WoL60	4.05	1.18	6.23	4.78	0.02	21.23	3.49	3.48
1WiL90	6.46	20.84	2.44	0.00	0.02	6.55	3.14	16.10
2WiL90	6.17	20.25	1.78	0.00	0.02	4.80	2.79	3.41
1WoL90	4.18	1.18	1.11	5.35	0.02	23.00	3.92	16.38
2WoL90	4.11	1.28	1.08	4.68	0.02	18.70	3.72	3.78

(1): 30 days after incubation; (2) 30 days after incubation with residual effect of liming. WoL30: without liming and 30% field capacity; WoL60: without liming and 60%; WoL90: without liming and 90%; WiL30: with liming and 30%; WiL60: with liming and 60%; and WiL90: with liming and 90%.

Table 4. Total accumulation of $C\text{-}CO_2$ and loss of $C\text{-}CO_2$ of *Organossolo Tiomórfico* after 30 days of incubation subjected to liming and different water depths.

Field Cap. (%)	$C\text{-}CO_2$-Total$^{(*)}$ ($g\ kg^{-1}$) WoL $^{(2)}$	WiL $^{(2)}$	$\bar{X}_1$	Field Cap. (%)	$C\text{-}CO_2$ emission$^{(**)}$ ($Mg\ ha^{-1}$) WoL $^{(2)}$	WiL $^{(2)}$	$\bar{X}_1$
30	0.27 bA	1.41 aA	0.84	30	0.73 bA	3.88 aA	2.31
60	0.29 bA	1.49 aA	0.89	60	0.79 bA	4.10 aA	2.45
90	0.22 bB	1.37 aA	0.79	90	0.59 bB	3.79 aA	2.19
$\bar{X}_2$	0.26	1.42		$\bar{X}_2$	0.71	3.92	
CV%		8.50		CV%		8.50	

Means followed by the same lowercase letter in the row do not indicate differences in limestone application. Equal uppercase letter in the column does not indicate differences between the water depths. $^{(ns)}$Absence of significance. $\bar{X}_1$: Overall mean of water depth; $\bar{X}_2$: Overall mean of limestone application. $^{(*)}$Total $C\text{-}CO_2$ data after 30 days of incubation converted from mg 500 g^{-1} to g kg^{-1}. $^{(**)}$Total $C\text{-}CO_2$ emission calculated based on bulk density, soil depth and total $C\text{-}CO_2$ contents. WoL30: without liming and 30% field capacity; WoL60: without liming and 60%; WoL90: without liming and 90%; WiL30: with liming and 30%; WiL60: with liming and 60%; and WiL90: with liming and 90%. $^{(1)}$ ANOVA without data transformation + Tukey test ($p < 0.05$). $^{(2)}$ ANOVA with data transformation + Tukey test ($p < 0.05$). $^{(3)}$ Kruskal-Wallis test + Fisher's least significant difference for liming factor levels and Wilcoxon test for drainage factor levels ($p < 0.05$).

The quantified data of $C\text{-}CO_2$ emission ranged from 0.59 to 4.10 Mg ha^{-1}, and the highest values were verified in the treatments of WiL60, WiL30 and WiL90 (4.10, 3.88 and 3.79 Mg ha^{-1}, respectively), reflecting greater loss of C by diffusion of $C\text{-}CO_2$ in the study area after the simulation of the first phase of the experiment. The lowest values of $C\text{-}CO_2$ emission were observed in the treatment of WoL90 (0.59 Mg ha^{-1}).

When evaluating a natural area in South Africa for 12 months, researchers observed $C\text{-}CO_2$ emission of 28.10 Mg ha^{-1}. However, when evaluating an area with perennial cultivation (citrus), the authors found higher cumulative CO_2 emission values, reaching 36.10 Mg ha^{-1} (MUNJONJI et al., 2024). This study found, as reported here, that agricultural practices significantly increase C losses from the soil to the atmosphere, particularly when these practices are poorly managed.

Attributes of humified organic matter after the second incubation

Despite the widespread use of liming, there is a significant gap of knowledge about its long-term effects on SOM mineralization (XIAO et al., 2024). In view of this, we performed the evaluations again for another 30 days after a period of soil rest, to investigate the residual effect of liming associated with different levels of drainage. As for the TOC and HA contents and values of the HA/TOC and E4/E6 ratios, these were not influenced by the factors evaluated in this second phase of the experiment (Table 5).

In the FA fraction, the highest mean values were found in the treatments that received $CaCO_3$ (WiL) and with greater soil drainage (30 and 60% field capacity) (Table 5). When evaluating the effect of the simulation of the different drainage levels on the C of this fraction, we observed the lowest mean values of FA in the wetter samples (90% field capacity). The same was observed for the FA/TOC ratio. These results corroborate the data observed in this fraction during the first phase of the experiment (Table 2), reinforcing the harmful effect of the increase in soil water content on FA contents.

Table 5. Organic matter attributes of *Organossolo Tiomórfico* after 30 days of incubation subjected to the residual effect of liming and different water depths.

Field Cap. (%)	TOC ($g\ kg^{-1}$)		
	WoL	WiL	$\bar{X}_1$ (1)
30	91.89	88.87	90.38 ns
60	88.28	88.43	88.35
90	93.71	88.17	90.94
$\bar{X}_2$ (1)	91.29 ns	88.49	
CV%		6.10	

Field Cap. (%)	FA ($g\ kg^{-1}$)		
	WoL	WiL	$\bar{X}_1$ (1)
30	13.22	13.82	13.52 A
60	12.17	15.36	13.76 A
90	9.77	12.01	10.89 B
$\bar{X}_2$ (1)	11.72 b	13.73 a	
CV%		13.10	

Field Cap. (%)	HA ($g\ kg^{-1}$)		
	WoL	WiL	$\bar{X}_1$ (1)
30	24.87	23.03	23.94 ns
60	22.43	24.71	23.57
90	24.93	21.57	23.25
$\bar{X}_2$ (1)	24.07 ns	23.10	
CV%		13.70	

Field Cap. (%)	FA/TOC (%)		
	WoL (2)	WiL (2)	$\bar{X}_1$
30	14.50 aA	15.80 aA	15.20
60	13.80 aA	17.50 aA	15.60
90	10.40 bB	13.60 aA	12.00
$\bar{X}_2$	12.90	15.60	
CV%		15.90	

Field Cap. (%)	HA/TOC (%)		
	WoL	WiL	$\bar{X}_1$ (1)
30	27.40	26.00	26.70 ns
60	25.50	28.00	26.80
90	26.60	24.50	25.60
$\bar{X}_2$ (1)	26.50 ns	26.10	
CV%		17.00	

Field Cap. (%)	HA/FA		
	WoL	WiL	$\bar{X}_1$ (1)
30	1.90	1.70	1.80 AB
60	1.80	1.60	1.70 B
90	2.60	1.80	2.20 A
$\bar{X}_2$ (1)	2.10 a	1.70 b	
CV%		17.80	

Field Cap. (%)	E4/E6		
	WoL	WiL	$\bar{X}_1$ (1)
30	3.30	3.40	3.30 ns
60	3.20	3.30	3.20
90	3.20	3.40	3.30
$\bar{X}_2$ (1)	3.20 ns	3.30	
CV%		6.60	

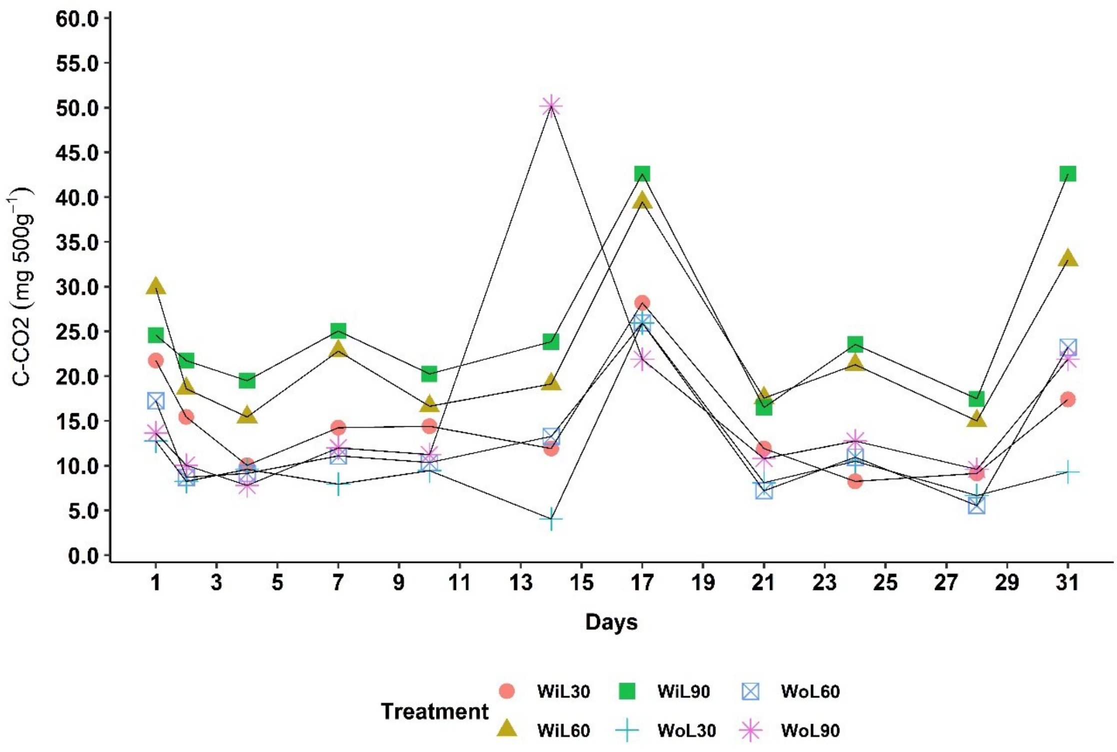
Means followed by the same lowercase letter in the row do not indicate differences in limestone application. Equal uppercase letter in the column does not indicate differences between the water depths. (ns)Absence of significance. $\bar{x}_1$: Overall mean of water depth; $\bar{x}_2$: Overall mean of limestone application. (1) ANOVA without data transformation + Tukey test ($p < 0.05$). (2) ANOVA with data transformation + Tukey test ($p < 0.05$). (3) Kruskal-Wallis test + Fisher's least significant difference for liming factor levels and Wilcoxon test for drainage factor levels ($p < 0.05$). WoL30: without liming and 30% field capacity; WoL60: without liming and 60%; WoL90: without liming and 90%; WiL30: with liming and 30%; WiL60: with liming and 60%; and WiL90: with liming and 90%. TOC: Total organic carbon; FA: Carbon of the fulvic acid fraction; FA/TOC: Percentage of carbon in the fulvic acid fraction relative to TOC; HA: Carbon of humic acid fraction; HA/TOC: Percentage of carbon of the humic acid fraction relative to TOC; HA/FA: Ratio of carbon of the humic acid and fulvic acid fractions; and E4/E6: E4/E6 ratio.

On the other hand, the highest mean values of the HA/FA ratio were observed in the treatments without liming (WoL) compared to those with liming (WiL), signaling the residual effect of $CaCO_3$ application on the more complex and stable fraction of humic substances, although at a lower intensity. It is worth pointing out that, in all treatments, the HA/FA ratio decreased when comparing the evaluation phases, while the E4/E6 ratio increased (Tables 2 and 5), suggesting a reduction in the aromaticity of SOM. Studies indicate that the HA fraction has a higher degree of aromaticity, complexity and stability than the FA fraction, which contains more carboxylic and phenolic groups, increasing the chelating capacity and cation exchange capacity of the soil (YANG et al., 2024).

Evolution of C-CO_2 after the second incubation

When evaluating soil respiration and CO_2 flux in samples of soils with organic constitution (±17% organic C) in Romania, Matei et al. (2023) observed a continuous trend of decrease in CO_2 losses. The same was observed in our study. The C-CO_2 values lost in this phase of the evaluation were considerably lower than those observed in the first phase, for all treatments (Figure 3). In most of the evaluations, the values quantified for the WiL treatments, regardless of the water depth, were higher than those of the WoL treatments, except for the evaluation performed on the 14th day, which showed a peak in microbial respiration for the WoL90 treatment.

Figure 4 shows an abrupt increase in C-CO_2 losses for the WoL90 treatment on the 14th day, followed by a decrease on the 17th day, when the other treatments showed a significant increase. This acceleration in the decomposition of SOM, which intensifies C-CO_2 losses, is known as the priming effect (FENG; ZHU, 2021). This effect is associated with microbial activity, which is modulated by factors such as temperature, oxygen, microbial biomass, and the quantity and quality of available labile C (SILES et al., 2022). In the present study, it is possible that nutrients previously immobilized in the more stable fractions of SOM were released during the mineralization of organic matter, stimulating the priming effect and increasing CO_2 emissions on days 14 and 17.



WoL30: without liming and 30% field capacity; WoL60: without liming and 60%; WoL90: without liming and 90%; WiL30: with liming and 30%; WiL60: with liming and 60%; and WiL90: with liming and 90%.

Figure 4. Evolution of C-CO_2 emission from *Organossolo Tiomórfico* subjected to different water depths and residual effect of liming over 30 days.

In this second phase of evaluation, aiming to identify the residual effects of liming on the evolution of C-CO_2 in the soil, we observed C-CO_2 losses of 0.62 and 1.56 Mg ha^{-1}. The highest overall mean values of C-CO_2 emission were found in the treatments that received $CaCO_3$ application (1.26 Mg ha^{-1}) and with lowest soil drainage (1.28 Mg ha^{-1}). On the other hand, the lowest overall mean values of the attribute were quantified in the WoL treatments (0.80 Mg ha^{-1}) and the highest soil drainage (0.76 Mg ha^{-1}) (Table 6). This indicates that, even under conditions of lower oxygen availability, the residual effect of liming increases the decomposition rates of SOM with consequent loss of C-CO_2 from the soil.

Our results highlight the importance of management in *Organossolos*, given the high losses of C-CO_2 when exposed to the liming process. Thus, it is urgent that sustainable management of these soils be implemented in order to avoid their degradation, as their contribution to climate change mitigation and environmental preservation is of paramount importance (GLINA et al., 2024).

Table 6. Total accumulation of C-CO_2 and loss of C-CO_2 of *Organossolo Tiomórfico* after 30 days of incubation subjected to the residual effect of liming and different water depths.

Field Cap. (%)	C-CO_2-Total$^{(*)}$ (g kg^{-1}) WoL	WiL	$\bar{X}_1$ (2)	Field Cap. (%)	C-CO_2 emission$^{(**)}$ (Mg ha^{-1}) WoL	WiL	$\bar{X}_1$ (2)
30	0.23	0.33	0.28 C	30	0.62	0.90	0.76 C
60	0.29	0.48	0.38 B	60	0.79	1.33	1.06 B
90	0.36	0.56	0.46 A	90	1.00	1.56	1.28 A
$\bar{X}_2$ (2)	0.29 b	0.46 a		$\bar{X}_2$ (2)	0.80 b	1.26 a	
CV%		13.60		CV%		13.60	

Means followed by the same lowercase letter in the row do not indicate differences in limestone application. Equal uppercase letter in the column does not indicate differences between the water depths. (ns)Absence of significance. $\bar{x}_1$: Overall mean of water depth; $\bar{x}_2$: Overall mean of limestone application. (*)Total C-CO_2 data after 30 days of incubation subjected to the residual effect of liming converted from mg 500 g^{-1} to g kg^{-1}. (**)Total C-CO_2 emission calculated based on bulk density, soil depth and total C-CO_2 contents. WoL30: without liming and 30% field capacity; WoL60: without liming and 60%; WoL90: without liming and 90%; WiL30: with liming and 30%; WiL60: with liming and 60%; and WiL90: with liming and 90%. (1) ANOVA without data transformation + Tukey test ($p < 0.05$). (2) ANOVA with data transformation + Tukey test ($p < 0.05$). (3) Kruskal-Wallis test + Fisher's least significant difference for liming factor levels and Wilcoxon test for drainage factor levels ($p < 0.05$).

Attributes evaluated throughout the experiment (treatments × incubation periods)

The data related to soil attributes quantified throughout the experiment were evaluated by principal component analysis (PCA). Table 7 presents the values of correlation of each variable with the axes of the principal component (PC). Correlation values $-0.70 \geq r \geq 0.70$ (high correlation) were selected to compose the PCA. After the initial treatment of the original data, the multivariate analyses showed accumulated variance for PCs 1 and 2 of 87.4% (Figure 5).

Table 7. Principal Component Analysis (PCA) matrix. Relative contribution corresponds to the Pearson's correlation (r) between each principal component (PC, axis) and the variables of the two incubation periods.

Variables	PC1	PC2
pH	0.23	0.88
Ca	0.20	0.93
Mg	0.02	0.03
Al	−0.10	−0.97
Na	−0.24	−0.15
K	−0.35	−0.62
P	0.94	−0.22
H+Al	−0.11	−0.94
FA	−0.87	0.42
FA/TOC	−0.84	0.43
HA	−0.94	−0.12
HA/TOC	−0.92	−0.12
HA/FA	0.36	−0.77
TOC	−0.50	0.01
E4/E6	−0.86	0.32
C-CO_2-Total	0.70	0.62

Bold values: Indicate high correlation ($-0.70 \geq r \geq 0.70$). pH: Active acidity; Ca: Exchangeable calcium; Mg: Exchangeable magnesium; Al: Exchangeable aluminum; Na: Exchangeable sodium; K: Exchangeable potassium; P: Available phosphorus; H+Al: Potential acidity; TOC: Total organic carbon; FA: Carbon of the fulvic acid fraction; FA/TOC: Percentage of carbon in the fulvic acid fraction relative to TOC; HA: Carbon of the humic acid fraction; HA/TOC: Percentage of carbon of the humic acid fraction relative to TOC; HA/FA: Ratio of carbon of the humic acid and fulvic acid fractions; and E4/E6: E4/E6 ratio.

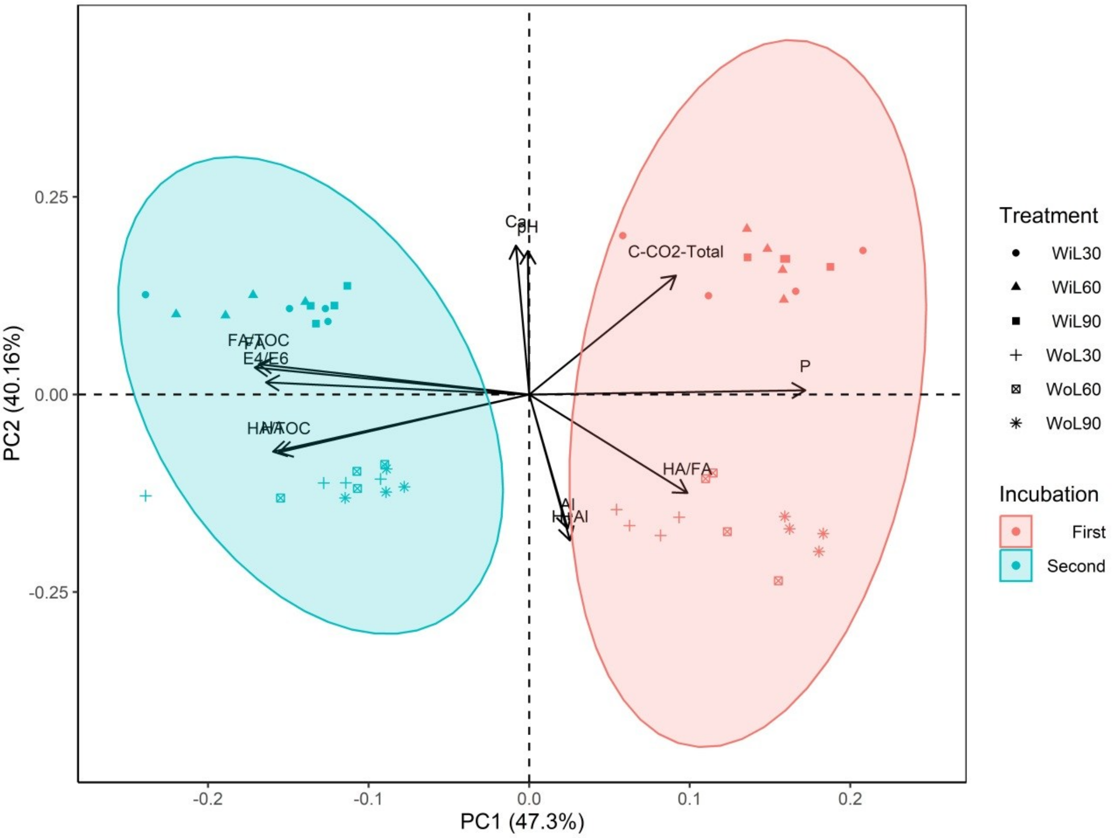


Figure 5. Principal component analysis (PCA) integrating the variables related to the chemical attributes of *Organossolo Tiomórfico* from the southeastern region of Brazil subjected to different water depths and residual effect of liming throughout the experiment.

In the PCA, a clear separation of the treatments was observed through the formation of four different groups, namely: (1) group formed by the WiL30, WiL60 and WiL90 treatments of the first phase of the experiment (upper right quadrant); (2) group formed by the WiL30, WiL60 and WiL90 treatments of the second phase of the experiment (upper left quadrant); (3) group formed by the WoL30, WoL60 and WoL90 treatments of the second phase of the experiment (lower left quadrant); and (4) group formed by the WoL30, WoL60 and WoL90 treatments of the first phase of the experiment (lower right quadrant).

The main axis (PC1, 47.3%) was responsible for the separation of the phases of the experiment, having the following parameters as discriminant variables (high correlation, $-0.70 \geq r \geq 0.70$): Available P (0.94), C-CO$_2$-Total (0.70), FA/TOC (−0.84), E4/E6 (−0.86), FA (−0.87), HA/TOC (−0.92) and HA (−0.94). Regarding the secondary axis (PC2, 40.16%), the attributes exchangeable Al (0.97), exchangeable Ca (0.93), pH in water (0.88), HA/FA (−0.77) and H+Al (−0.94) were the variables that most contributed to the construction of this axis (Table 7). This axis was responsible for the separation of the treatments that received $CaCO_3$ application, regardless of the water depth.

According to the correlation matrix of the principal components, the first group of highly weighted variables implies that PC1 is primarily associated with P availability, the dynamics of stabilization, accumulation and distribution of humic fractions of SOM, and the total emission of mineralizable C. In PC2, the highly weighted variables indicate that the component is mainly related to the attributes of fertility and C mobility in the soil. It is worth pointing out that TOC did not show a high correlation with the axes of formation of the multivariate analysis, reinforcing the hypothesis that liming in association with drainage mainly affects the stabilization (by humification) and accumulation of SOM, and that the monitoring of this effect has to prioritize the investigation of SOM compartments, especially the functional, reactive and soluble ones.

Through the study of the PCA (Figure 5), it is possible to observe that the WiL30, WiL60 and WiL90 treatments of the first evaluation period are more associated with the highest contents of C-CO$_2$-Total (1.42 g kg^{-1}; Table 4). For the WoL30, WoL60 and WoL90 treatments also from the first phase of the experiment, these are more related to the higher values of exchangeable acidity and potential acidity (Table 3), as well as the lowest selective loss of the less soluble fraction of SOM (HA) (4.60; Table 2). The highest values of FA (13.73 g kg^{-1}), FA/TOC (15.60%) and E4/E6 (3.30) (Table 5) are more closely linked to the WiL30, WiL60 and WiL90 treatments in the second evaluation period, and the highest values of HA (24.00 g kg^{-1}) and HA/TOC (26.50%) (Table 5) correlate better with the WoL30, WoL60 and WoL90 treatments of the second phase of the experiment.

CONCLUSION

Liming and drainage modified the C contents of humic substances, microbial respiration rates and soil fertility attributes. There was a reduction of C in less complex fractions (FA) with less drainage and in more complex fractions (HA) with application of $CaCO_3$ combined with the water depth.

In the short term, liming increased the mineralizable C release by up to ten times compared to treatments without liming. Losses due to C-CO_2 emissions reached 4.10 Mg ha^{-1} in 30 days. Its residual effect also increased SOM mineralization, with additional losses of 1.56 Mg ha^{-1} after another 30 days of incubation.

The results of the present study indicate that liming, in the short and medium term, in areas of *Organossolos* considerably increases soil C-CO_2 losses.

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