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Chemical Fractions of Soil Organic Matter and Their Interactions with Cu, Zn and Mn in Vineyards in Southern Brazil

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Abstract

This study aimed to evaluate the impact of vineyard cultivation time and the use of metal-based fungicides on the chemical fractions of soil organic matter (SOM), as well as their interactions with Cu, Zn, and Mn in vineyard soils from Southern Brazil with varying histories of fungicide application. Soils samples were collected from vineyards aged 35, 37, and 39 years in the Serra Gaúcha region, and 13, 19, and 36 years in the Campanha Gaúcha. In each region, samples were also collected from a non-anthropized reference area. In the oldest vineyards, sampling was conducted both within and between the planting rows. From the collected soil samples, chemical fractionation of SOM was performed, yielding the following fractions: non-humic substances (nHS), particulate organic matter (POM), fulvic acid (FA), humic acid (HA), and humin (Hu). After this step, Fourier-transform infrared (FTIR) spectra were obtained for the humic acids, from which the aromaticity index (AI) and relative intensities (RI) were calculated. In each SOM fraction, total organic carbon and the concentrations of Cu, Zn, and Mn were determined. Changes in land use influenced the distribution and chemical nature of SOC and its interaction with metals. HA from vineyard soils showed greater humification and aromaticity, while Cu was mainly associated with HA and FA, and Zn and Mn accumulated in the Hu. Over time, vineyard cultivation, especially with cover crop management, contributed to increased SOC stocks, reaching levels comparable to those in native areas.

Keywords: heavy metals; *Vitis vinifera*; FTIR; fulvic acid; humic acid; pesticides

1. Introduction

Soil organic matter (SOM) plays a fundamental role in assessing soil quality, being considered the main indicator and the largest reservoir of organic carbon on the Earth's surface, with approximately 58% carbon in its composition [1]. Among its definitions, SOK is regarded as the most complex, dynamic, heterogeneous, and reactive component of soil [2]. Due to its heterogeneity, it comprises clusters of organic compounds with varying degrees of complexity, structural diversity and lability [3].

The chemical composition of soil organic matter (SOM) plays a critical role in governing its dynamics within the soil matrix [4]. Humic substances (HS)—primarily composed of humic acid

(HA), fulvic acid (FA), and humin (Hu)—constitute the most stable fraction of SOM and typically represent between 80% to 90% of the organic matter in mineral soils. Owing to their high reactivity and functional group diversity, HS possess a strong capacity to complex heavy metals in agroecosystems, thereby influencing the mobility, availability, and overall behavior of these elements in the soil environment [5–7].

The excessive use of metal-based fungicides has been identified as a primary factor contributing to the accumulation of metals such as copper (Cu) and zinc (Zn) in agroecosystems worldwide, particularly in vineyards [8–15]. This phenomenon is especially evident in major grape-producing countries such as France, Spain, Italy [16,17], and Brazil [8,18,19], where viticultural practices are heavily reliant on the application of agrochemicals [20,21]. Among the agrochemicals most commonly used in viticultural systems are fungicides [22], particularly those formulated with metals such as Cu and Zn [12,23,24].

In Brazil, the state of Rio Grande do Sul, located in the southern region of the country, comprises two traditional grape-producing areas: Serra Gaúcha and Campanha Gaúcha [19]. These regions are characterized by high relative humidity and significant rainfall throughout the grapevine growth cycle, which favor the development of fungal diseases [25–28]. As a result, grapevines are frequently subjected to successive applications of copper-based fungicides, such as Bordeaux mixture [$Ca(OH)_2$ + $CuSO_4$] and copper oxychloride [$CuCl_2{\cdot}3Cu(OH)_2$], as well as zinc and manganese compounds found in Mancozeb ($C_4H_6MnN_2S_4 \times Zn$) [29]. These repeated applications may lead to the accumulation of these metals in soils over time.

Another important point is that recent studies have shown that increasing Cu and Zn concentrations in vineyard soils can also lead to higher levels of available Mn, representing an additional contamination risk. This increase may result from Cu displacing Mn at soil colloid sorption sites, thereby enhancing Mn concentration in the soil solution and its uptake by grapevines [30]. Alternatively, the stress induced by elevated Cu and Zn levels may stimulate greater root exudation by plants, which in turn increases Mn availability in the rhizosphere by lowering the pH [31,32].

The interaction between SOM chemical compartments and metal ions can influence the mobility of both organic compounds and metals within the soil profile [33], either through the formation of low-molecular-weight organometallic complexes or via the mineralization of organic ligands, leading to the release of metals into the soil solution [34]. These organo-mineral interactions can thus affect SOM dynamics [4] and may be responsible for changes in both the quantity and chemical composition of SOM [33].

In this context, studies evaluating the forms, distribution, and accumulation of metals in the chemical fractions of SOM over time, as well as SOM quality under different climatic conditions, soil types, and vineyard management systems, are increasingly important for assessing their impact on agroecosystems [35], particularly in viticultural systems. These studies directly contribute to several United Nations Sustainable Development Goals (SDGs) [36], especially **SDG 12 (Responsible Consumption and Production),** by promoting the sustainable use of agricultural inputs; **SDG 13 (Climate Action)**, by considering the role of soils in carbon sequestration; and **SDG 15 (Life on Land), b**y aiming to conserve and sustainably manage terrestrial ecosystems. Therefore, the aim of this study was to evaluate the impact of vineyard cultivation time and the application of metal-based fungicides on the chemical fractions of SOM and their interactions with Cu, Zn, and Mn in vineyard soils from Southern Brazil with different histories of fungicide application.

2. Material and Methods

2.1. Characterization of Study Sites and Soil Sampling

The vineyard areas selected for this study are located in the Serra Gaúcha (municipality of Bento Gonçalves) and Campanha Gaúcha (municipality of Santana do Livramento) regions, in the state of Rio Grande do Sul, southern Brazil. The Serra Gaúcha region is situated at an altitude of 600 to 800 meters, with annual averages of 1,700 mm precipitation, 17.2°C temperature, and 76% relative

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humidity. The predominant soil in this area is classified as Litholic Entisol [37]. Conversely, the Campanha Gaúcha region is located at an altitude of 100 to 300 meters, recording annual averages of 1,370 mm precipitation, 18.4°C temperature, and 75% relative humidity [19]. The predominant soil in Campanha Gaúcha is a Sandy Typic Hapludalf [37]. In both regions, three vineyards were selected, each with distinct cultivation histories and, consequently, different application histories of Cu, Zn, and Mn-based fungicides.

In Serra Gaúcha, the chosen vineyards were V35 (35 years old, 29°09′50″S and 51°32′03″W), V37 (37 years old, 29°09′48″S and 51°31′45″O), and V39 (39 years old, 29°09′42″S and 51°31′44″O). Additionally, soil samples were collected from an adjacent forest area (F, 29°09′46″S and 51°31′49″O). In vineyards V35 and V37, soil was collected from the planting rows, whereas in V39, soil collection included both planting rows and inter-rows (V39IR). The cultivar present in all three vineyards was Isabel (*Vitis labrusca* L.), grafted on its own roots, and the training system used was a trellis. The main fungicides employed in these vineyards were Delan®, Captan®, Folpan®, Manzate®, and Curzate®, with 16 annual applications of these products, in addition to three applications of Bordeaux mixture. At the time of soil collection, fertilizers and acidity correctives had not been applied in the vineyards for five years.

In Campanha Gaúcha, the selected vineyards were V13 (13 years old, 30°46′39″S and 55°22′35″W), V19 (19 years old, 30°46′38″S and 55°21′59″W), and V36 (36 years old, 30°46′50″S and 55°21′07″W). A native grassland area (NG, 30°47′26″S and 55°22′04″W), adjacent to the vineyards, was also sampled. The cultivar in all vineyards was Cabernet Sauvignon (*Vitis vinifera*), grafted onto SO4 rootstock (*Vitis berlandieri* x *Vitis riparia*), and the training system was the Geneva Double Curtain (GDC). The predominant plant species found in the native grassland area included *Paspalum notatum*, *Paspalum plicatulum*, *Desmodium incanum*, *Ageratum conyzoides* L., *Chevreulia acuminata* Less, and *Cyperus brevifolius*. In vineyards V13 and V19, soil was collected from the planting rows, whereas in vineyard V36, collection encompassed both planting rows and inter-rows (V36IR). In each vineyard, 9.0 kg ha^{-1} $year^{-1}$ of copper sulfate and 8.0 kg ha^{-1} $year^{-1}$ of copper hydroxide were applied, totaling 8.8 kg Cu ha^{-1} $year^{-1}$.

The decision to collect soil from the oldest vineyards in each region, from both planting rows and inter-rows, is justified by the presence of cover crops in the inter-rows, which favor the immobilization of these metals in SOM. Conversely, in the planting rows, spontaneous vegetation is desiccated, promoting SOM mineralization and, consequently, maintaining these metals in more labile fractions in the soil. Thus, the oldest vineyards were selected to allow for this differentiation, as the distinctions would be more evident in areas with a long history of viticultural management [38].

In Serra Gaúcha, soil samples were collected in July 2017 at depths of 0.00-0.05, 0.05-0.10, 0.10-0.15, and 0.15-0.20 m. In Campanha Gaúcha, soil samples were collected at layers of 0.00-0.05, 0.05-0.10, 0.10-0.20, and 0.20-0.40 m. In all areas, soil samples were collected from six points per area [39].

2.2. Preparation of Soil Samples and Chemical Analyses

Following collection, soil samples underwent air-drying, grinding, and sieving through a 2 mm mesh. Clay content was quantified using the pipette method [40]. The total organic carbon (TOC) content was determined with an auto-analyzer (LECO, TruSpec CHNS, USA). Standard soil analysis procedures for southern Brazil [41] were followed to determine several key parameters: pH in water (1:1 soil-water ratio), SMP index, available concentrations of P, K, Cu, Zn, and Mn (extracted via Mehlich-1), and exchangeable concentrations of Al, Ca, and Mg (extracted with 1.0 mol L^{-1} KCl). Subsequently, available P content in the resulting solution was measured colorimetrically using a UV-Visible Spectrophotometer (UV – 1600, PRO-TOOLS). Potassium (K) content was determined by flame photometry (DM-62, DIGIMED). Aluminum (Al) values were obtained through titration with 0.0125 mol L^{-1} NaOH. Concentrations of Ca, Mg, Cu, Zn, and Mn were quantified using an Atomic Absorption Spectrophotometer (Aanalyst 200, PERKIN ELMER). Based on these analytical results, calculations were performed for H+Al content, potential ($CEC_{pH7.0}$) and effective ($CEC_{ef.}$) cation exchange capacities, saturation of $CEC_{pH7.0}$ by Ca+Mg+K, and Al saturation [42] (Table A1).

Another portion of the collected soil was refrigerated at −4°C and subsequently subjected to chemical fractionation of SOM [43], yielding the following fractions: (i) non-humic substances (HCl-extractable); (ii) particulate organic matter (POM); (iii) fulvic acid (FA); (iv) humic acid (HA); and (v) humin (Hu). For the separation of POM (density <1.0 g cm^{-3}) and simultaneous extraction of non-humic substances, 50 mL of 0.1 mol L^{-1} HCl were added to 5.0 g of soil from Bento Gonçalves and 10.0 g of soil from Santana do Livramento. The suspension was mechanically shaken (120 rpm) for 2 hours. It was then centrifuged (10 min, 3,500 rpm), and the supernatant was filtered through a 0.45 µm paper membrane. This procedure was repeated three times. The POM retained on the filter was weighed for quantification and then discarded, while the extract containing the non-humic substances (HCl 0.1 mol L^{-1}) was measured for volume and stored at 4°C.

Subsequently, for the chemical fractionation of HS, 50 mL of 0.5 mol L^{-1} NaOH solution were added to the soil sample, and the suspension was mechanically shaken for 3 hours, followed by centrifugation (10 min, 3,500 rpm). This procedure was repeated until the supernatant became clear. The total volume of the alkaline extract (AE) was then measured, and an aliquot of approximately 35 mL was stored at 4°C. The remaining solution had its pH adjusted to 2.0 using 4.0 mol L^{-1} HCl, and the suspension was left to stand for 24 hours. After acidification, part of the carbon in the extract precipitated, corresponding to the HA fraction, while the remaining carbon stayed in suspension, corresponding to the FA fraction. The precipitated HA was separated from the FA by centrifugation (10 min, 1,500 g), and an aliquot of approximately 35 mL of FA was stored at 4°C. The organic matter insoluble in NaOH and remaining bound to the soil mineral matrix corresponds to the Hu fraction.

The recovered HA was subjected to purification to remove mineral particle residues using a 5% HF/HCl solution under mechanical shaking for 2 hours, repeated three times, yielding the purified solid HA fraction. The remaining mass (purified HA) was washed with distilled water for 30 minutes and centrifuged (5 min, 1,500 g), with this step repeated five times. Finally, the samples were oven-dried at 60°C under forced air circulation [44].

Fourier-transform infrared (FTIR) spectra were obtained for the HA fraction from all areas in both locations; however, analyses were limited to the 0.00–0.05 m and 0.15–0.20 m soil layers in Bento Gonçalves, and the 0.00–0.05 m and 0.20–0.40 m layers in Santana do Livramento. The surface layer (0.00–0.05 m) was selected due to its greater exposure to copper- and zinc-based fungicide applications, the influence of vegetation, and the accumulation of agricultural residues over the years. The deepest layer in each site was analyzed to evaluate the behavior of this SOM fraction in subsurface conditions. HA samples were analyzed using Fourier-transform infrared spectroscopy (FTIR) (Shimadzu 8300), prepared as KBr pellets (1:100), with 32 scans and a resolution of 4 cm^{-1} across the spectral range of 4000 to 400 cm^{-1}.

The assignment of absorption bands was performed [45,46]. Based on the FTIR spectra, the aromaticity index (AI) was calculated [47], using Equation 1:

$$\text{IA}\ (cm^{-1}) = \frac{I_{C=C}}{I_{C-H}}, \qquad (1)$$

where $I_{C=C}$ is the absorption intensity around 1630 cm^{-1}; and I_{C-H} is the absorption intensity around 2920 cm^{-1}, after baseline correction between 1800–1500 cm^{-1} and 3000–2800 cm^{-1}.

The relative intensities (RI) of the main absorption bands were calculated according to Gerzabek et al. (2006), by dividing the corrected intensity of each given peak (2900, 1715, 1630, 1540, 1400, 1235, and 1080 cm^{-1}) by the sum of the intensities of all peaks, and multiplying by 100. The parameters used to determine peak intensities were defined by base1/peak/base2 (cm^{-1}): 3000/2900/2800; 1800/1715/1500; 1800/1620/1500; 1800/1540/1500; 1500/1430/900; 1500/1250/900; and 1500/1080/900.

The total soil organic carbon (SOC) contents were determined using an elemental analyzer (LECO TruSpec CHNS) at 1000 °C, at the Nutrient Cycling Laboratory (LCN) of the Center for Nuclear Energy in Agriculture (CENA), University of São Paulo (USP), in Piracicaba, São Paulo, Brazil. Total Cu, Zn, and Mn contents were calculated as the sum of the chemical fractions obtained [48]

The SOC contents in the liquid extracts were determined by adding 2 mL of each sample—HCl extract (C_{HCl}), alkaline extract (C_{EA}), and fulvic acid (C_{FA})—and 1 mL of 0.4 mol L^{-1} acidified potassium dichromate to 10 mL test tubes, followed by heating at 60 °C for 4 hours. The absorbance was then measured at 580 nm using a UV–visible spectrophotometer (Model UV-5300PC, Power Supply, China) [49].

Cu, Zn, and Mn concentrations in the HCl (Cu_{HCl}, Zn_{HCl}, and Mn_{HCl}), alkaline (Cu_{EA}, Zn_{EA}, and Mn_{EA}), and fulvic acid fractions (Cu_{FA}, Zn_{FA}, and Mn_{FA}) were determined by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES; Perkin Elmer, Optima 2100 DV). For the Hu fraction, total C (C_{Hu}) was determined using the elemental analyzer (LECO TruSpec CHNS), and total Cu (Cu_{Hu}), Zn (Zn_{Hu}), and Mn (Mn_{Hu}) were determined by the digestion with HF + $HClO_4$ [48], followed by measurement using Atomic Absorption Spectroscopy (AAS; Perkin Elmer, AA200, USA).

2.3. Calculations and Statistical Analysis

The C (C_{HA}), Cu (Cu_{HA}), Zn (Zn_{HA}), and Mn (Mn_{HA}) contents in the HA fraction were determined using Equation 2:

$$M_{HA} = M_{EA} - M_{FA} \qquad (2)$$

where M_{HA} is the Cu, Zn, or Mn content in the HA fraction; M_{EA} is Cu, Zn, or Mn content in the EA; M_{FA} is the Cu, Zn, or Mn content in the FA fraction.

The ratios of HA/FA and (HA+FA)/HU were calculated based on the C content in the humic fractions of SOM.

The Aromaticity Index (AI) was calculated as the ratio between IR_{1620}/IR_{2920}.

The contents of TOC, C, Cu, Zn, and Mn in the humic (FA, HA, and HU), non-humic (HCl), and particulate (POM) fractions of SOM were subjected to a homoscedasticity test (F-max test). When variances were homogeneous, the data were analyzed using parametric tests in two situations: pairwise comparisons between areas were performed using the Student's t-test (LSD), and comparisons between soil layers were performed using Tukey's test ($p < 0.05$). All analyses were conducted using Sisvar software (version 5.6).

The data were standardized and subjected to Principal Component Analysis (PCA) using R software (version 3.6.2) [50] with the packages "FactorMinerR" [51] and "factoextra" [52], to assess the similarity among total contents and the chemical fractions of SOM for Cu, Zn, and Mn, as well as clay content and soil pH (in water). An additional PCA was also performed using the C and metal data associated with HA, including the FTIR variables.

3. Results

3.1. TOC Contents and C Associated with Humic Fractions of SOM

In the Serra Gaúcha region, vineyard cultivation time favored the formation of POM and C_{HA} fractions in the 0.00–0.05 m layer, with the highest contents observed in vineyard areas. For C_{HCl}, in the 0.00–0.05 m layer, the highest contents were found in the F and in vineyards V35 and V37, while in deeper layers, the highest values were also observed in F and in the oldest vineyard areas (V39 and V39IR). For C_{FA}, the highest contents were consistently observed in F, both at the surface and at depth, whereas V39EL showed the lowest C_{FA} contents across all evaluated layers. Cu_{Hu} and TOC showed similar trends: in the 0.00–0.05 m layer, the highest contents were observed in V37, followed by F. In the deeper layers, the highest contents were generally found in F and V35. Regarding the sampled soil layers, for all evaluated areas, the contents of POM, C_{HCl}, C_{FA}, C_{HA}, C_{Hu}, and TOC were highest in the 0.00–0.05 m layer, decreasing with soil depth (Table 1).

In the Campanha Gaúcha areas, the highest POM contents were observed in the V19 site across all evaluated soil layers. Regarding C_{HCl} levels, the highest values were recorded in the V36IR site, also across all layers. For the C_{FA} and C_{HA} fractions, the vineyards generally showed higher contents than the NG in the 0.00–0.05 m layer. In the deeper layers, the NG site presented higher C_{FA} contents compared to the vineyard areas. For C_{Hu} and TOC, no significant differences were found between the reference area and the vineyards. With increasing depth, all evaluated areas generally showed higher contents of POM, C_{HCl}, C_{FA}, C_{HA}, C_{Hu}, and TOC in the 0.00–0.05 m layer, with decreasing levels observed in deeper layers (Table 1).

Table 1. Total organic carbon contents in soil organic matter chemical fractions in vineyards with different cultivation times in the Serra and Campanha Gaúcha Regions.

Camada, m	Serra Gaúcha					CV, %	Camada, m	Campanha Gaúcha					CV, %
	F	V35	V37	V39	V39IR			NG	V13	V19	V36	V36IR	
	mg kg^{-1}							mg kg^{-1}					
	POM												
0.00-0.05	7.93^{ns} C	12.02 aBC	17.81 aA	13.01 aB	15.55 aAB	18.18	0.00-0.05	4.19 aC	5.43 aBC	9.01 aA	4.48 aC	6.35 aB	11.63
0.05-0.10	7.40 C	8.86 abB	7.21 cC	7.33 bC	11.90 abA	2.99	0.05-0.10	3.91 aA	2.79 bC	3.45 bAB	3.31 bABC	3.11 bBC	10.32
0.10-0.15	7.90 B	7.79 bB	9.38 bA	7.37 bB	6.73 bB	6.00	0.10-0.20	2.95 bB	2.63 bB	3.38 bA	2.78 bB	2.73 bB	8.06
0.15-0.20	7.67 ABC	8.53 abAB	8.84 bA	7.05 bC	7.11 bBC	10.32	0.20-0.40	2.96 bAB	2.84 bAB	3.17 bA	2.84 bAB	2.59 bB	10.29
CV,%	4.16	15.08	3.81	14.41	20.87			8.49	11.74	14.03	12.39	5.76	
	C_{HCl}												
0.00-0.05	0.54 aA	0.48 aA	0.55 aA	0.43 aB	0.31 aB	9.70	0.00-0.05	0.14 aB	0.13 ^{ns}B	0.14 aB	0.13 ^{ns}B	0.17 aA	9,57
0.05-0.10	0.28 bA	0.19 bB	0.31 bA	0.35 bA	0.16 bB	17.07	0.05-0.10	0.12 bA	0.09 B	0.09 bB	0.09 B	0.11 bA	6,54
0.10-0.15	0.28 bB	0.17 bC	0.20 bE	0.32 bA	0.17 bCD	6.40	0.10-0.20	0.12 b^{NS}	0.10	0.07 b	0.12	0.10 b	28,87
0.15-0.20	0.19 cB	0.13 bCD	0.18 bBC	0.27 bA	0.11 bD	17.76	0.20-0.40	0.10 cAB	0.11 AB	0.08 bB	0.10 AB	0.11 bA	16,95
CV,%	9.19	10.40	18.42	7.94	13.57			3.77	18.61	14.13	29.29	7.70	
	C_{FA}												
0.00-0.05	2.96 aA	2.59 aA	2.50 aA	2.56 aA	1.19 aB	13.92	0.00-0.05	0.98 aB	1.65 aA	1.83 aA	0.70 aB	1.93 aA	18.23
0.05-0.10	1.56 bB	0.64 bC	1.34 bB	1.92 bA	0.42 abC	13.31	0.05-0.10	0.54 bA	0.48 bA	0.52 bA	0.25 bB	0.50 bA	20.69
0.10-0.15	1.22 bA	0.37 cC	1.03 cB	0.59 cCD	0.18 bD	12.97	0.10-0.20	0.51 bA	0.45 bAB	0.49 bAB	0.26 bC	0.36 bBC	16.46
0.15-0.20	0.72 cB	0.44 bcD	0.84 cA	0.26 cC	0.08 bE	3.80	0.20-0.40	0.26 bA	0.28 cA	0.20 bAB	0.15 bB	0.28 bA	20.43
CV,%	10.49	9.16	8.28	11.85	66.90			19.78	8.08	36.11	15.20	12.53	
	C_{HA}												
0.00-0.05	2.57 aC	6.08 aAB	7.04 aA	4.95 aB	5.01 aB	16.16	0.00-0.05	0.58 abC	2.40 aB	2.13 ^{ns}B	3.15 aB	5.07 aA	27.74
0.05-0.10	3.00 aB	0.72 bD	2.65 bB	3.65 abA	1.24 bC	12.58	0.05-0.10	0.89 aB	1.08 b B	1.06 B	1.81 bA	1.73 bA	12.00
0.10-0.15	0.79 bC	0.94 bC	1.89 cB	3.46 abA	0.96 bC	12.39	0.10-0.20	0.38 abC	1.19 bAB	1.02 B	1.47 bA	1.33 bA	15.45
0.15-0.20	0.82 bB	1.06 bB	1.73 cA	2.10 bA	0.64 bB	18.90	0.20-0.40	0.61 bB	1.13 bA	0.75 B	1.41 bA	1.31 bA	17.96
CV,%	18.04	11.53	8.14	21.89	24.87		CV,%	22.93	7.26	62.86	15.36	11.40	

F = Forest; V35 = Vineyard with 35 years of cultivation; V37 = Vineyard with 37 years of cultivation; V39 = Vineyard with 39 years of cultivation; V39IR = Vineyard with 39 years of cultivation, with soil collected from the inter-row; NG = Native grassland; V13 = Vineyard with 13 years of cultivation; V19 = Vineyard with 19 years of cultivation; V36 = Vineyard with 36 years of cultivation; V36IR = Vineyard with 36 years of cultivation, with soil collected from the inter-row; POM = Particulate organic matter; Chcl = Carbon from non-humic substances; C_{FA}

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= Carbon from fulvic acids; C_{HA} = Carbon from humic acids; C_{Hu} = Carbon from humin. Means followed by the same lowercase letter in the column do not differ significantly by Tukey's test ($p < 0.05$). Means followed by the same uppercase letter in the row do not differ significantly by t-test (LSD) ($p < 0.05$). ns = not significant.

3.2. Carbon Distribution in the Humic Fractions of SOM

In the Serra Gaúcha regions, the highest proportions of C in the F were observed in the Hu, with percentages of 82, 71, 64, and 54% in the 0.00–0.05, 0.05–0.10, 0.10–0.15, and 0.15–0.20 m layers, respectively (Figure 1a, b, c, d). However, with the cultivation of vineyards over the years, there was a reduction in the proportion of C_{Hu}, ranging from 74% in V37 to 33% in V39 in the 0.00–0.05 m layer (Figure 1a). On the other hand, the percentage of C_{HA} increased, especially in V39 and V39IR, with values of 16% and 10%, respectively, in the 0.00–0.05 m layer, following the same increasing pattern in the other layers. Comparing the original contents of C_{HA} in the F (2.57 mg kg^{-1}) to V39 and V39IR (4.95 and 5.01 mg kg^{-1}, respectively), this increase was on average 93%. It is important to highlight that with increasing vineyard cultivation time, the percentage of C_{Hu} decreased, while the percentage of POM increased, especially at depth (Figure 1).

The Campanha Gaúcha region showed a similar pattern across all evaluated layers for the NG area. The most abundant C fraction was C_{Hu}, with average percentages of 75% across all evaluated layers (Figure 1e, f, g, h). In the 0.00–0.05 m layer, an increase in the percentage of POM was observed, especially in the V19 area (41%). Additionally, in all evaluated layers, a small portion of the C was distributed in the C_{FA} and C_{HA}, mainly in the older vineyards. While in the NG area, C accumulated in the FA and HA fractions at proportions of 2% and 3%, respectively, in the vineyard areas this accumulation reached 6% and 16% in V36IR, respectively (0.00–0.05 m) (Figure 1e).

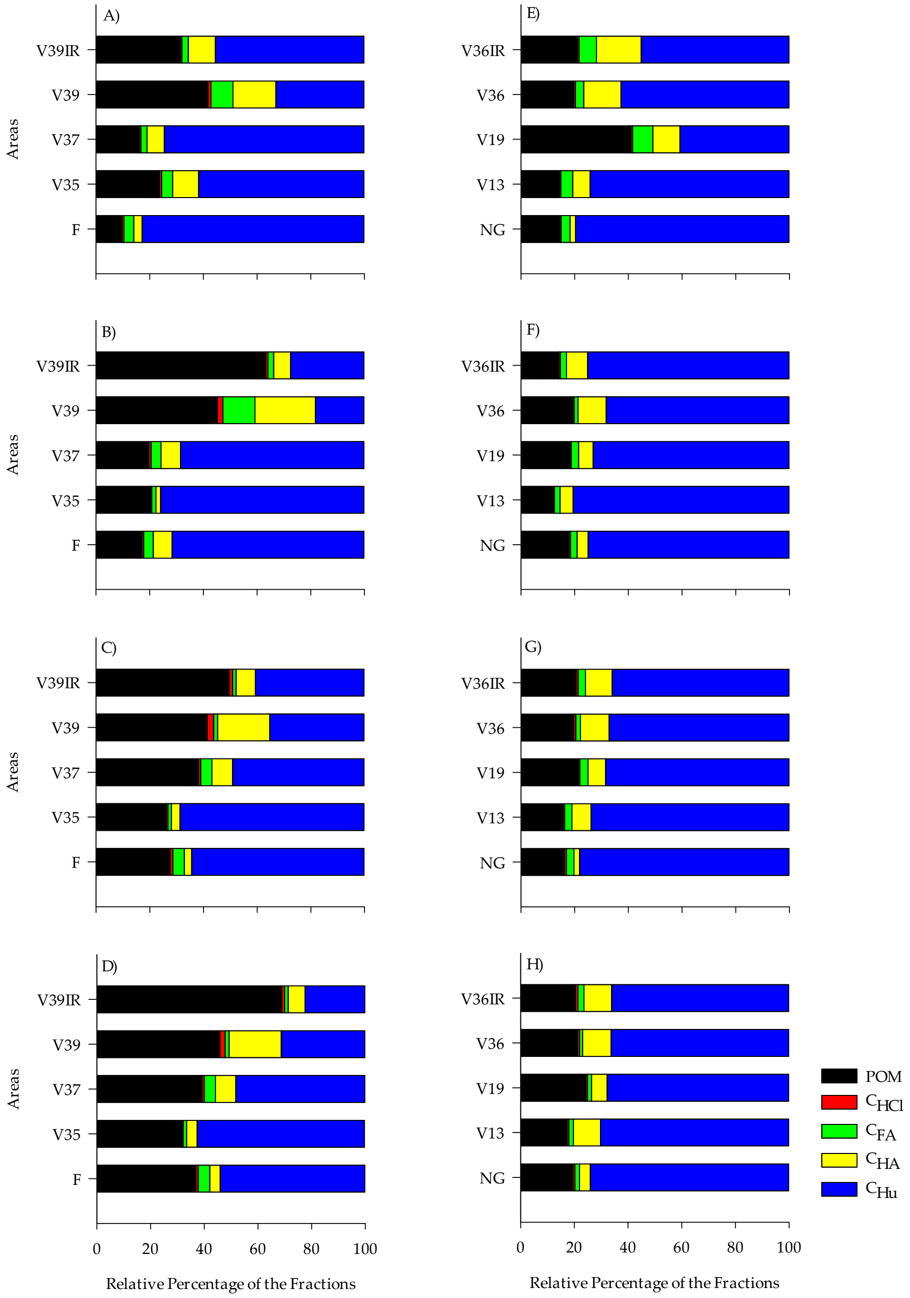

Figure 1. Percentage distribution of C in the chemical fractions of SOM for the 0.00–0.05 m (A), 0.05–0.10 m (B), 0.10–0.15 m (C), and 0.15–0.20 m (D) layers in the Serra Gaúcha region, and for the 0.00–0.05 m (E), 0.05–0.10 m (F), 0.10–0.20 m (G), and 0.20–0.40 m (H) layers in the Campanha Gaúcha region. F: Forest; V35: Vineyard with 35 years of cultivation; V37: Vineyard with 37 years of cultivation; V39: Vineyard with 39 years of cultivation; V39IR: Vineyard with 39 years of cultivation, with soil collected from the inter-row; NG: Native grassland; V13: Vineyard with 13 years of cultivation; V19: Vineyard with 19 years of cultivation; V36: Vineyard with 36 years of cultivation; V36IR: Vineyard with 36 years of cultivation, with soil collected from the inter-row; POM = Particulate organic matter; C_{HCl} = Carbon associated with non-humic substances; C_{FA} = Carbon in the fulvic acid fraction; C_{HA} = Carbon in the humic acid fraction; C_{Hu} = Carbon in the humin fraction.

3.3. C_{HA}/C_{FA} and (C_{HA}+C_{FA})/C_{Hu} Ratios

In both the Serra and Campanha Gaúcha regions, the vineyard areas showed C_{HA}/C_{FA} ratios higher than 1.0 in all evaluated layers. Moreover, especially in the vineyards of the Campanha Gaúcha region, there was an increase in the C_{HA}/C_{FA} ratio with increasing depth. In the reference areas, C_{HA}/C_{FA} ratio values were lower than 1.0 in the 0.00–0.05 m and 0.10–0.15 m layers in the F area, and in the 0.00–0.05 m and 0.10–0.20 m layers in the NG area (Table 2).

Table 2. Ratios between the humic fractions of soil organic matter in vineyards with different cultivation durations in the Serra Gaúcha and Campanha Gaúcha regions.

Layer, m	Serra Gaúcha				
	F	V35	V37	V39	V39IR
	mg kg^{-1}				
	C_{HA}/C_{FA}				
0.00-0.05	0.88	2.26	2.82	1.95	5.35
0.05-0.10	1.93	1.12	2.00	1.94	3.02
0.10-0.15	0.66	2.51	1.85	5.86	5.76
0.15-0.20	1.14	2.42	2.06	3.53	9.16
	$(C_{FA}+C_{HA})/C_{Hu}$				
0.00-0.05	0.08	0.21	0.12	0.74	0,23
0.05-0.10	0.15	0.04	0.16	2.56	0,27
0.10-0.15	0.11	0.06	0.25	0.59	0,21
0.15-0.20	0.13	0.09	0.24	0.55	0,32

Layer, m	Campanha Gaúcha				
	NG	V13	V19	V36	V36IR
	mg kg^{-1}				
	C_{HA}/C_{FA}				
0.00-0.05	0.61	1.46	1.30	4.55	2.66
0.05-0.10	1.81	2.31	2.07	7.42	3.56
0.10-0.20	0.74	2.70	2.24	6.24	3.68
0.20-0.40	2.38	5.94	3.87	10.49	4.96
	$(C_{FA}+C_{HA})/C_{Hu}$				
0.00-0.05	0.07	0.15	0.84	0.27	0.45
0.05-0.10	0.09	0.09	0.11	0.18	0.14
0.10-0.20	0.06	0.13	0.14	0.19	0.19
0.20-0.40	0.08	0.17	0.11	0.18	0.19

F: Forest; V35: Vineyard with 35 years of cultivation; V37: Vineyard with 37 years of cultivation; V39: Vineyard with 39 years of cultivation; V39IR: Vineyard with 39 years of cultivation, with soil collected from the inter-row; NG: Native grassland; V13: Vineyard with 13 years of cultivation; V19: Vineyard with 19 years of cultivation; V36: Vineyard with 36 years of cultivation; V36IR: Vineyard with 36 years of cultivation, with soil collected from the inter-row; C_{HA}/C_{FA}: Ratio between the carbon in the HA and FA; $(C_{HA} + C_{FA})/C_{Hu}$ = Ratio between the sum of the carbon in the HA and FA and the carbon in the Hu.

For the $(C_{FA} + C_{HA})/C_{Hu}$ ratio, the reference areas showed the lowest values when compared to the vineyard areas, except for V35 (Table 2).

3.4. Molecular Composition Assessed by FTIR and RI

The FTIR spectra of the HA from all evaluated areas and depths showed similar patterns (Figure 2). The main absorption bands identified and their respective assignments were: bands at 3400 cm^{-1}, attributed to OH groups; two bands in the 2900 and 2850 cm^{-1} region, corresponding to aliphatic C–H stretching; a band at 1720–1715 cm^{-1}, due to carboxylic C=O stretching; a band in the 1630–1620 cm^{-1} region, related to aromatic C=C stretching; bands around 1540 cm^{-1}, attributed to N–H bending and C–N stretching; bands at 1400 cm^{-1}, due to aliphatic C–H bending; bands around 1235 cm^{-1}, attributed to C–O stretching and OH deformation from carboxylic groups; bands at 1080–1050 cm^{-1}, corresponding to C–O stretching in polysaccharides; and a band at 1044–1026 cm^{-1}, attributed to Si–O vibrations from inorganic material that was not removed during HF purification [53,54] (Figure 2).

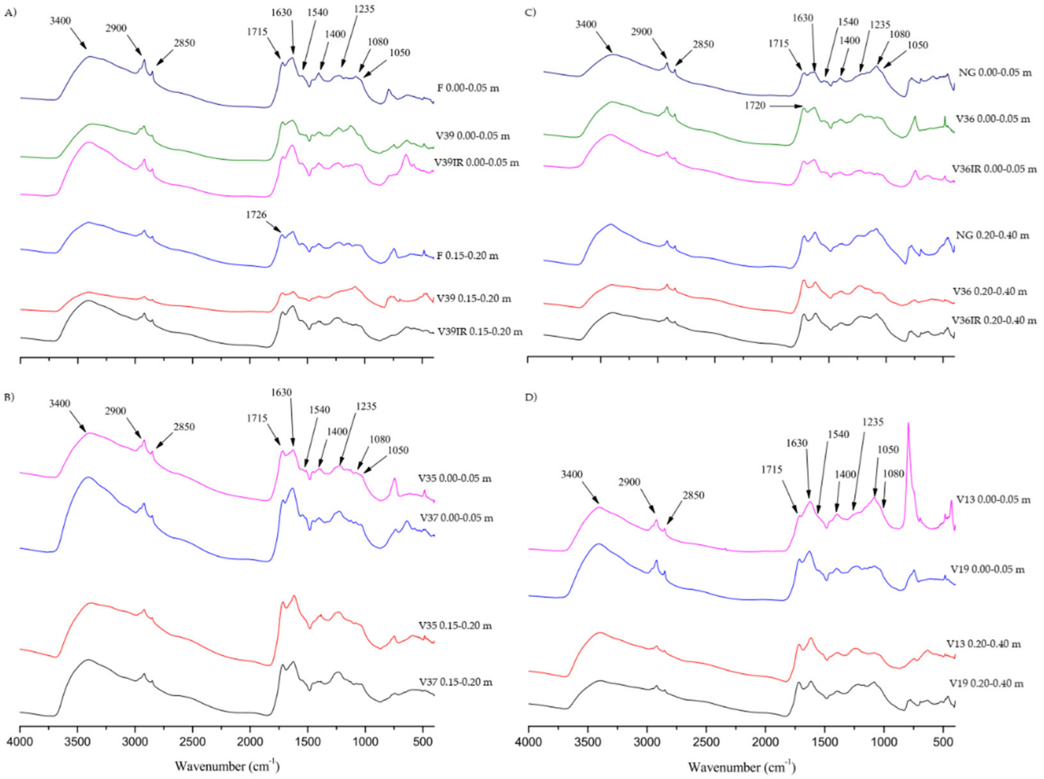

Figure 2. Pattern of HA spectra in soils from the Serra Gaúcha (A and B) and Campanha Gaúcha (C and D) regions, in vineyard soils with varying organic matter and clay contents, and conducted under different training systems with a history of metal-based fungicide applications. **A:** F = Forest; V39 = Vineyard with 39 years of cultivation; V39IR = Vineyard with 39 years of cultivation, with soil collected from the inter-row areas in the municipality of Bento Gonçalves. **B:** V35 = Vineyard with 35 years of cultivation; V37 = Vineyard with 37 years of cultivation, in the municipality of Bento Gonçalves. **C:** NG = Native grassland; V36 = Vineyard with 36 years of cultivation; V36IR = Vineyard with 36 years of cultivation, with soil collected from the inter-row areas in the municipality of Santana do Livramento; V13 = Vineyard with 13 years of cultivation; V19 = Vineyard with 19 years of cultivation, in the municipality of Santana do Livramento.

In the Serra Gaúcha areas, the relative intensity (RI) of aliphatic groups (RI_{2920}) ranged from 5.84% to 11.44%. The highest values in the surface layer were observed in the F and V35 areas, and in all evaluated areas, the values decreased with depth. In the soils of the Campanha Gaúcha region, RI ranged from 4.80% to 11.80%. The highest values were found in the surface layer in the NG and V19 areas, and similar to the Serra Gaúcha region, these values decreased with depth in all evaluated areas (Table 3).

Table 3. Relative intensity of the FTIR bands of SOM fractions and aromaticity index in soils from vineyards with different cultivation durations and in reference areas of the Serra Gaúcha and Campanha Gaúcha regions.

Area	Layer, m	Relative intensity of the FTIR bands							AI
		RI_{2920}	RI_{1715}	RI_{1620}	RI_{1540}	RI_{1400}	RI_{1235}	RI_{1080}	
		------------------------- % -------------------------							
		Serra Gaúcha							
F	0.00-0.05	11.4	22.9	22.6	8.8	9.8	11.4	13.1	1.9
	0.15-0.20	7.7	24.2	23.7	10.1	8.7	13.0	12.6	3.1
V35	0.00-0.05	11.3	22.9	23.2	8.9	9.6	10.9	13.3	2.1
	0.15-0.20	5.8	28.0	26.5	7.0	8.6	13.6	10.5	4.5
V37	0.00-0.05	7.7	25.2	26.9	10.1	7.3	12.9	9.8	3.5
	0.15-0.20	6.7	26.8	25.5	9.8	6.7	15.2	9.4	3.8

V39	0.00-0.05	8.9	23.9	21.9	7.7	7.7	13.4	16.,6	2.5
	0.15-0.20	6.4	20.6	18.4	7.1	7.1	16.3	24.1	2.9
V39IR	0.00-0.05	9.4	21.3	25.9	10.8	9.4	10.8	12.3	2.8
	0.15-0.20	7.6	22.9	24.6	10.2	12.2	12.7	9.8	3.2
				Campanha Gaúcha					
NG	0.00-0.05	10.6	18.9	17.3	3.4	8.9	15.6	25.1	1.6
	0.20-0.40	6.3	20.4	19.9	7.2	6.8	15.4	23.9	3.2
V13	0.00-0.05	9.0	18.9	23.8	9.0	9.0	9.8	20.5	2.6
	0.20-0.40	5.4	27.0	27.0	7.6	7.6	13.5	11.9	5.0
V19	0.00-0.05	11.1	21.8	24.2	8.7	9.1	11.5	13.5	2.2
	0.20-0.40	4.8	16.0	17.6	5.6	7.2	16.8	32.0	3.7
V36	0.00-0.05	9.7	24.5	21.8	8.8	7.4	13.9	13.9	2.2
	0.20-0.40	7.6	28.4	22.5	7.1	8.3	15.4	10.7	2.9
V36IR	0.00-0.05	9.1	25.2	25.2	7.7	7.7	13.3	11.9	2.8
	0.20-0.40	5.5	24.1	20.1	7.0	7.0	16.1	20.1	3.6

F: Forest; V35: Vineyard with 35 years of cultivation; V37: Vineyard with 37 years of cultivation; V39: Vineyard with 39 years of cultivation; V39IR: Vineyard with 39 years of cultivation, with soil collected from the inter-row; NG: Native grassland; V13: Vineyard with 13 years of cultivation; V19: Vineyard with 19 years of cultivation; V36: Vineyard with 36 years of cultivation; V36IR: Vineyard with 36 years of cultivation, with soil collected from the inter-row; RI: Relative intensity; FTIR: Fourier-transform infrared spectroscopy; AI: aromaticity index.

The RI of carboxylic groups (RI_{1715}) in the Serra Gaúcha areas ranged from 20.57% to 26.79% across the evaluated layers. In this region, there was a slight increase with depth in most areas, except for V39. In the Campanha Gaúcha areas, the RI of this band ranged from 16.00% to 27.03%, and as in the Serra Gaúcha region, there was an increase in RI with depth, except for V19 and V36IR (Table 3).

The RI of aromatic groups (RI_{1620}) ranged from 18.44% to 26.92% in the Serra Gaúcha region and from 17.32% to 27.03% in the Campanha Gaúcha region. In the Serra Gaúcha areas, a decrease in RI with depth was observed in V37, V39, and V39IR, while in F and V35, RI increased with depth. In the Campanha Gaúcha region, RI increased with depth in NG, V13, and V36, while in the other areas, V19 and V36IR, there was a decrease with depth. Regarding the AI, an increase in values with depth was observed in all areas. When comparing the reference areas with the vineyard areas, it was noted that with increased cultivation time, there was an increase in AI values at the surface in the vineyard areas (Table 3).

In both the Serra Gaúcha and Campanha Gaúcha regions, the RI of nitrogen-containing compounds (RI_{1540}) decreased with depth in the vineyard areas, while in the reference areas (F and NG), it increased. In the Serra Gaúcha region, values ranged from 7.00% to 10.83%, and in the Campanha Gaúcha region, from 3.35% to 9.02%. For carboxylate groups (RI_{1400}), a decrease in RI with depth was observed, except in V39IR (Serra Gaúcha) and V36 (Campanha Gaúcha). In the Serra Gaúcha region, values ranged from 6.70% to 12.29%, and in the Campanha Gaúcha region, from 6.79% to 9.13% (Table 3).

The RI of the C–O stretching from carboxyl groups (RI_{1235}) in the Serra Gaúcha region ranged from 10.33% to 16.81%, with the highest values found in the 0.00–0.05 m layer. In the Campanha Gaúcha region, values ranged from 9.84% to 16.08%. In this region, lower values were observed at the surface and higher values at depth, except in NG (Table 3).

The RI_{1080} values ranged from 9.38% to 24.11% in the Serra Gaúcha region and from 10.65% to 32.00% in the Campanha Gaúcha region. In both evaluated regions, a decrease in RI_{1080} values with depth was observed as vineyard cultivation time increased, when compared to the reference areas. Regarding depth, a general decrease in values was observed, except for the V39 area in Serra Gaúcha and V36IR in Campanha Gaúcha, where an increase with depth occurred (Table 3).

3.5. Distribution of Cu, Zn, and Mn in the Humic Fractions of SOM

In the Serra Gaúcha region, the distribution of Cu in SOM fractions in F showed that Cu tends to accumulate in the Cu_{Hu} fraction, representing up to 78% of the total Cu content in the 0.00–0.05 m layer (Figure 3). The same trend was observed in the 0.10–0.15 m and 0.15–0.20 m layers, with approximately 73% in both layers. In the 0.05–0.10 m layer, the highest Cu accumulation in the reference area occurred in the HA and FA fractions, accounting for 49% and 33%, respectively. In vineyard areas, Cu also tends to accumulate in the Cu_{Hu} fraction, with an average distribution of 70% across the vineyard sites, reaching up to 96% in the oldest vineyard area. In the V35 and V37 areas, a decrease in Cu contents in the Cu_{FA} and Cu_{HA} fractions was observed with depth, dropping from 38% to 5% and from 23% to 6%, respectively (Figure 3). In the Campanha Gaúcha region, the highest percentages of the Cu_{HCl} fraction were recorded in the NG, representing 3% of the total Cu, decreasing in vineyard areas, where it averaged 0.25% in the 0.00–0.05 m layer. Across the evaluated layers, the Cu_{HCl} fraction in NG decreased with depth, averaging 1.8% of the total Cu, while in vineyard areas the opposite trend was observed, averaging 0.6% of the total Cu. For the Cu_{FA} fraction, the highest contents were found in NG, accounting for 29% of the total, decreasing in vineyard areas, where it averaged 19%. The opposite trend was observed for the Cu_{HA} fraction, with higher contents in vineyard areas, representing 42% of the total Cu, compared to 14% in NG. The highest percentages in NG, V37, and V39 were found in the Cu_{Hu} fraction, representing 46%, 42%, and 46%, respectively, in the surface layer (Figure 3). Further details on Cu contents in SOM chemical fractions are presented in Table A2.

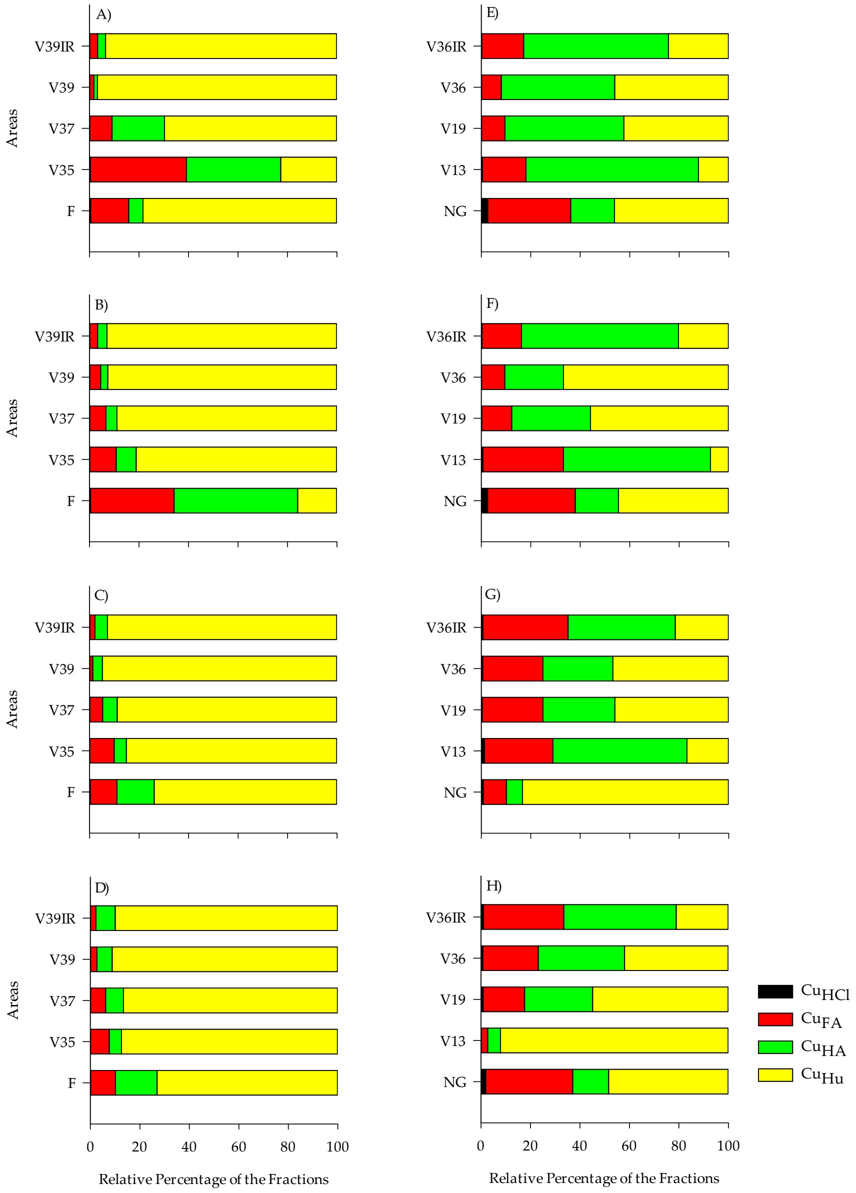

Figure 3. Percentual distribution of Cu in the chemical fractions of SOM for the layers 0.00–0.05 m (A), 0.05–0.10 m (B), 0.10–0.15 m (C), and 0.15–0.20 m (D) in the areas of the Serra Gaúcha region, and 0.00–0.05 m (E), 0.05–0.10 m (F), 0.10–0.20 m (G), and 0.20–0.40 m (H) in the areas of the Campanha Gaúcha region. F: Forest; V35: Vineyard with 35 years of cultivation; V37: Vineyard with 37 years of cultivation; V39: Vineyard with 39 years of cultivation; V39BL: Vineyard with 39 years of cultivation, with soil collected between the planting lines; NG: Native grassland; V13: Vineyard with 13 years of cultivation; V19: Vineyard with 19 years of cultivation; V36: Vineyard with 36 years of cultivation; V36BL: Vineyard with 36 years of cultivation, with soil collected between the planting lines; Cu_{HCL}: Copper associated with non-humic substances; Cu_{FA}: Copper in the FA fraction; Cu_{HA}: Copper in the HA fraction; Cu_{Hu}: Copper in the Hu fraction.

In the Serra Gaúcha region, the distribution of Zn among SOM chemical fractions shows that, for all areas evaluated, the highest proportion of Zn occurred in the Zn_{HCl} and Zn_{Hu} fractions (Figure 4). It is important to note that Zn contents in the Zn_{FA} and Zn_{HA} fractions were below the detection limit of the equipment (**Table A3**). In the reference area, Zn preferentially accumulated in the Hu, representing 92% of the total Zn in the surface layer and averaging 87% in the deeper layers. In the vineyard areas, the opposite trend was observed. Over time, vineyard cultivation led to a redistribution of Zn fractions from the Zn_{Hu} to the Zn_{HCl} fraction, with 17% in areas V35, V37, and

V39, and up to 90% in the V39IR area (0.00–0.05 m). In deeper layers, the proportions slightly decreased compared to the reference area, with Zn_{Hu} representing an average of 82% of the total Zn in areas V35, V37, and V39, and 10% in the V39IR area in the surface layer (Figure 4). In the Campanha Gaúcha region, the highest Zn percentages were found in the Zn_{Hu} fraction, representing 87% of the total Zn at the surface and over 94% in deeper layers. For area V35 (0.00–0.05 m), the highest Zn contents were observed in the Zn_{HCl} fraction, representing 66% of the total Zn, while in deeper layers, the Zn_{Hu} fraction accounted for more than 90% of the total Zn. In areas V37, V39, and V39IR, Zn_{HCl} contents were not detected. No Zn_{FA} or Zn_{HA} contents were detected in any of the areas evaluated. Thus, for areas V37, V39, and V39IR, all Zn was accumulated in the Zn_{Hu} fraction (Figure 4). Further details on Zn contents in SOM chemical fractions are presented in Table A3.

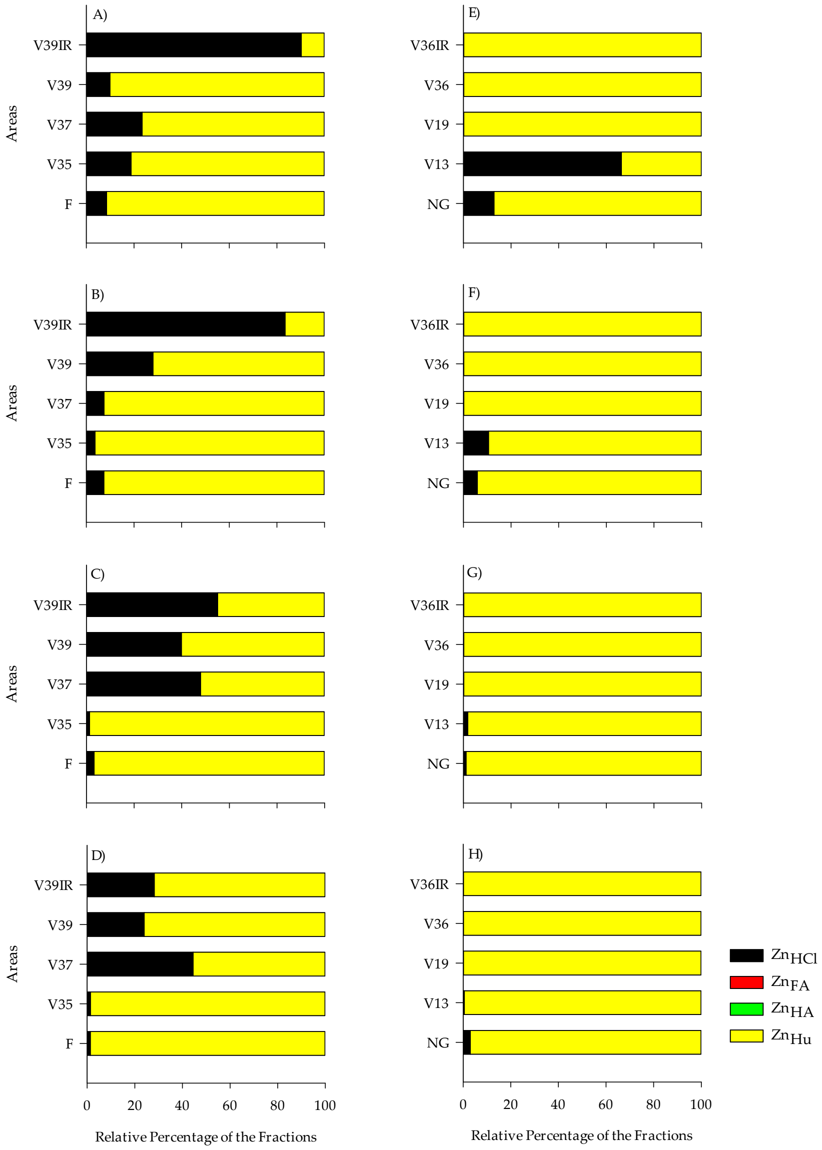

Figure 4. Percentual distribution of Zn in the chemical fractions of SOM for the layers 0.00–0.05 m (A), 0.05–0.10 m (B), 0.10–0.15 m (C), and 0.15–0.20 m (D) in the areas of the Serra Gaúcha region, and 0.00–0.05 m (E), 0.05–0.10 m (F), 0.10–0.20 m (G), and 0.2 0–0.40 m (H) in the areas of the Campanha Gaúcha region. F: Forest; V35: Vineyard with 35 years of cultivation; V37: Vineyard with 37 years of cultivation; V39: Vineyard with 39 years of cultivation; V39BL: Vineyard with 39 years of cultivation, with soil collected between the planting lines; NG: Native grassland; V13: Vineyard with 13 years of cultivation; V19: Vineyard with 19 years of cultivation; V36: Vineyard with 36 years of cultivation; V36BL: Vineyard with 36 years of cultivation, with soil collected between the planting lines; Zn_{HCL}: Zinc associated with non-humic substances; Zn_{FA}: Zinc in the FA fraction; Zn_{HA}: Zinc in the HA fraction; Zn_{Hu}: Zinc in the Hu fraction.

The assessment of Mn distribution shows that the Mn_{HCl} fraction tends to increase in vineyard areas (averaging 12%) compared to the reference area (7%) in the surface layer. It was observed that, for both vineyards and the reference area, these higher percentages tend to decrease with depth, although remaining higher in F (5%) than in the vineyards (3%). For the Mn_{FA} fraction, cultivation time tends to reduce the percentages of this fraction compared to the reference area, while for the Mn_{HA} fraction, the opposite occurs. The highest percentages for all evaluated areas were found in the Mn_{Hu} fraction, with 88% in the reference area and an average of 82% in vineyard areas in the surface layer. In deeper layers, there is an increase in these percentages for all areas evaluated (Figure 5). In the Campanha Gaúcha region, the highest Mn accumulation percentages were observed in the Mn_{Hu} fraction, representing 69% in the NG area and 84% in vineyard areas. Both in NG and in the vineyard areas, there is a trend of increasing percentages of this fraction with depth, reaching 82% in NG and 95% in vineyard areas. In areas NG, V35, and V37, the highest Mn_{HCl} accumulations occur at the surface and tend to decrease with depth. For areas V39 and V39IR, Mn_{HCl} was not detected. No Mn_{FA} percentages were detected in any of the evaluated areas. For the Mn_{HA} fraction, an increase in Mn percentages was observed in vineyard areas (6%) compared to NG (3.5%) at the surface, with the same trend observed in deeper layers (Figure 5). Further details on Mn contents in the chemical fractions of soil organic matter are presented in Table 4.

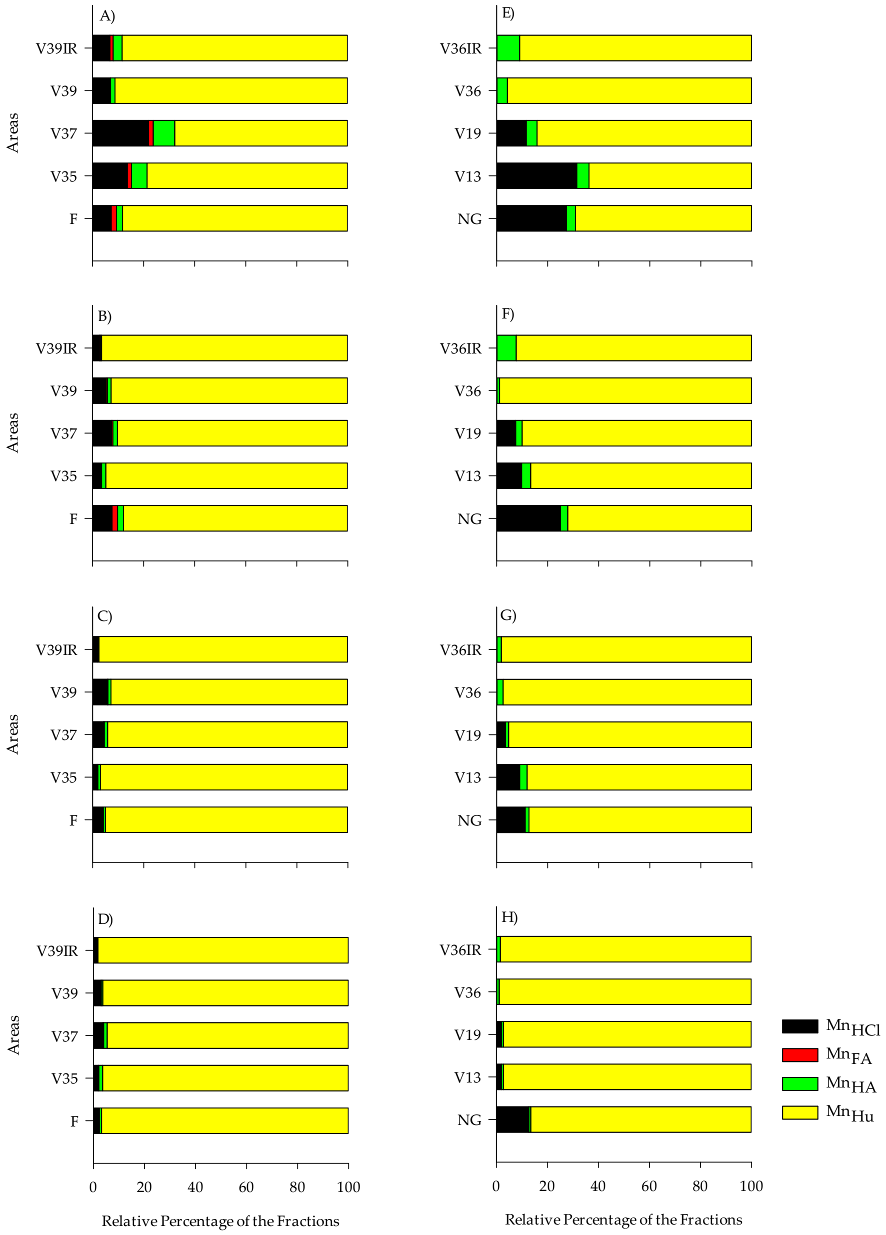

Figure 5. Percentage distribution of Mn in the chemical fractions of SOM for the 0.00–0.05 m (A), 0.05–0.10 m (B), 0.10–0.15 m (C), and 0.15–0.20 m (D) layers in the Serra Gaúcha region, and for the 0.00–0.05 m (E), 0.05–0.10 m (F), 0.10–0.20 m (G), and 0.20–0.40 m (H) layers in the Campanha Gaúcha region. F: Forest; V35: Vineyard with 35 years of cultivation; V37: Vineyard with 37 years of cultivation; V39: Vineyard with 39 years of cultivation; V39BL: Vineyard with 39 years of cultivation, with soil collected between the planting lines; NG: Native grassland; V13: Vineyard with 13 years of cultivation; V19: Vineyard with 19 years of cultivation; V36: Vineyard with 36 years of cultivation; V36BL: Vineyard with 36 years of cultivation, with soil collected between the planting lines; Mn_{HCL}: Manganese associated with non-humic substances; Mn_{FA}: Manganese in the FA fraction; Mn_{HA}: Manganese in the HA fraction; Mn_{Hu}: Manganese in the Hu fraction.

4. Discussion

4.1. TOC Contents and C Associated with Humic Fractions of SOM

Data on POM contents show that vineyard management over the years increased the levels of this fraction when compared to the reference areas. POM is the light fraction of SOM and is mainly composed of animal and plant residues, root fragments, fungal hyphae, and spores [55]. In viticultural agroecosystems, it can originate from vine plant residues such as leaves, fruits, stems, and bark, or even from cover crops after their annual senescence [56]. Changes in POM are closely related to shifts in land use or soil management, as POM is considered a labile and sensitive fraction that responds quickly to management-induced modifications and is susceptible to microbial attack in the soil. However, changes in its stocks promoted by soil management are usually observed in the short term [57]. In the evaluated areas, it was observed that over the years—at least 35 years in the Serra Gaúcha region and 13 years in the Campanha Gaúcha region—there was an increase of 52% to 96% and 7% to 115%, respectively, in POM contents in vineyard areas compared to the reference areas. This indicates that these timeframes were sufficient to restore and increase the levels of this fraction in these soils. This happens because viticultural agroecosystems have characteristics that, unlike annual crops, make them favorable for long-term C storage under proper management regimes. These plants are perennial, their cultivation involves little or no soil tillage, and cover crops managed between the vine rows can contribute to C inputs in the soil [58–60].

The C contents extracted from vineyard soils at different depths using 0.1 mol L^{-1} HCl represent the hydrophilic, low molecular weight organic fraction soluble in acidic medium, which is weakly bound to the mineral fraction. These are called non-humic substances (C_{HCl}) and are considered labile compounds with rapid mineralization in the soil, as they are readily used as substrates by soil microorganisms [61]. The observed increase in C_{HCl} contents in the vineyards may indicate higher microbiological activity in the soil, largely due to the history of nutrient and C inputs from fertilization, whether mineral, organic, or green manure, since this fraction is related to organic compounds derived from SOM decomposition [62]. Furthermore, organic compounds (such as sugars, polyphenols, amino acids, carbohydrates) extracted by HCl, considered non-humic substances, serve as energy sources for the microbial population due to their easy degradability, contributing to high microbial activity in the soil [63]. However, from an environmental standpoint, this is not desirable. If metals accumulate in these fractions—as occurs with Zn and Mn, for example (Tables A3 and A4)—there may be an increased toxicity potential from these elements in these agroecosystems, since these compounds are easily degraded and increase their concentrations in solution, as well as their uptake by cultivated plants.

Higher contents of FA, especially in vineyard areas, may be related to the recent addition of plant material (leaves and branches), which initially increase the FA content in the soil [64]. However, in the long term, a higher prevalence of fulvic acids may result in organic matter losses through percolating water [65]. According to these authors, this occurs because FA are the group with the lowest molecular weight and the highest number of phenolic and carboxylic groups, showing greater solubility and polarity than HA, and consequently greater mobility in the soil. Nevertheless, this increase in FA contents is beneficial to cultivated plants [66,67], since it enhances soil fertility due to the high density of charges they present, such as carboxylic and phenolic groups.

Regarding HA, the increased contents in vineyard areas can be explained by the deposition of plant residues (leaves and branches), which favor the accumulation of this fraction in the soil over the years. Along with the high precipitation occurring in the study regions, Serra Gaúcha and Campanha Gaúcha, this intensifies the biodegradation of more labile organic matter fractions, resulting in a faster transformation of FA into HA [68].

The increase in the $C_{FA} + C_{HA}$ contents, when compared to the reference areas across all evaluated layers, indicates greater reactivity of the functional groups of SOM. In the case of HA, their main functions are attributed to phenolic and carboxylic functional groups [69], including the ability to complex heavy metals [70]. Regarding HA, we observe a slight increase in the RI of the bands corresponding to carboxylic groups (1715 cm^{-1}) and the C–O stretching of carboxyl groups (1235 cm^{-1}), indicating that with longer vineyard management time, the reactivity of these functional groups increases in these areas (Figure 2).

The highest absolute contents of C_{Hu}, in general, were observed in the surface layers of the reference areas, except for area V37 in the Serra Gaúcha and area V13 in the Campanha Gaúcha, following the same pattern observed for TOC contents. These results can be attributed to the absence of anthropogenic action in both areas and the higher input of litterfall in the F and the addition of native plant material and rhizodeposition in the NG. In natural environments, the formation of HS is associated with microbial activity, with humification over time being the result of both biological and abiotic processes [71]. Similar results were reported in vineyards established in the highlands of Santa Catarina state, Brazil, where the highest C_{Hu} contents were found in vineyard areas, while the highest TOC contents were recorded in forest areas [72]

SOM has been suggested as a key indicator of soil quality, whether based on total contents, soil carbon stocks, or the distribution of its fractions when compared to nearby non-anthropized areas [73]. It was observed that land use change for vineyard establishment over the years led to a reduction in TOC contents in the surface layer (0.00–0.05 m) by 61.67% in Serra Gaúcha and by 21.41% in Campanha Gaúcha in the oldest vineyards, except for V37 in the 0.00–0.05 m layer. This difference in the magnitude of TOC decrease between the evaluated regions is mainly due to soil characteristics and the original TOC levels in the reference areas (Table A1). Campanha Gaúcha had lower original TOC contents compared to Serra Gaúcha, meaning there was less carbon available for mineralization in Campanha Gaúcha. The higher absolute C_{Hu} contents in area V37 in Serra may also be related to the higher TOC and clay contents found in these soils. C_{Hu} accumulation tends to occur when SOM levels are higher, and in addition, the higher clay contents in this area may have contributed to a greater degree of SOM humification and increased C_{Hu} levels, highlighting the effect of soil texture on SOM stabilization [72].

When evaluating the diagnostic layer of 0.00–0.20 m for fruit crops [42], these losses reached 54.41% and 19.20%, respectively. This indicates that grapevine cultivation tends to reduce soil carbon contents, mainly due to management practices and soil disturbance during vineyard establishment, which break down soil aggregates and increase the release of previously protected organic matter [74,75]. As a result, there is greater SOM mineralization compared to reference areas [73,76]. Another factor that can reduce SOM levels and protection is the removal of natural vegetation under the grapevines using herbicides [77].

However, soil management using conservation techniques, such as no-tillage, cover cropping, and organic fertilization, can increase TOC levels in vineyards. This effect can be observed when comparing TOC contents in older vineyard areas, where soil was sampled both within the rows—without cover crop management (i.e., where cover crops or spontaneous vegetation were removed)—and between the rows—where cover crops were maintained. Cover crops resulted in an increase of 59.4% and 37.9% in TOC content in the Serra Gaúcha and Campanha Gaúcha regions, respectively, in the 0.00–0.05 m layer. These findings are consistent with other studies reported in the literature [78,79]. In a study aiming to evaluate the effect of cover crops on TOC content in vineyards, the authors observed that treatments with cover crops (grasses, legumes, and cover crop mixtures) had, on average, 15.6% more TOC compared to the control treatment without cover crop management [80].

The C_{HA}/C_{FA} ratio reflects the mobility of SOM [81]. When this ratio presents values close to 1, it indicates the presence of high-quality organic material that can improve soil physical properties and plant growth. Conversely, when the ratio exceeds 1, it suggests a loss of C from the more labile FA, a situation commonly observed in sandy soils. In this study, the higher C_{HA}/C_{FA} ratios observed in vineyard areas indicate greater persistence of FA in the reference areas. The obtained ratio suggests a high humification rate in the vineyard areas compared to the reference sites. From a soil quality perspective, this is highly beneficial. Humification is the process through which organic matter is transformed into more stable and complex compounds that remain in the soil for long periods. This leads to several benefits, such as improved soil structure, enhanced soil fertility, increased water retention capacity, and greater microbial activity in the soil. Another factor that may contribute to the higher C_{AH}/C_{AF} ratios is the elevated CEC levels observed in the vineyard areas, especially in the Campanha Gaúcha region, which promote the humification process [82].

The $(C_{HA}+C_{FA})/C_{Hu}$ ratio can provide useful information about SOM loss along the soil profile. Values below 1 indicate a higher proportion of carbon in the Hu and are associated with a strong interaction between SOM and the mineral phase of the soil, resulting in high SOM stability in the more recalcitrant Hu [81,83].

The RI_{2920} decreased with soil depth in both the Serra Gaúcha and Campanha Gaúcha regions, possibly due to the greater contribution of plant residues (aboveground parts and roots) from spontaneous vegetation, cover crops, or pruning remains to the lipid fraction at the soil surface. This reduction in aliphatic groups (RI_{2920}) with depth, observed in all evaluated areas, may also be related to the limited mobility of these compounds in the soil profile due to their hydrophobic nature [84,85]. A slight increase in RI_{1715} values was also observed in the evaluated areas of Serra Gaúcha and Campanha Gaúcha, probably associated with increased functionalization of HA at greater soil depths [86]. The increase in aromatic groups (RI_{1620}) with soil depth can be attributed to a relative concentration effect resulting from the selective degradation of more labile compounds and the translocation of aromatic compounds along the soil profile. This process is possible due to the functionalization of aromatic rings, which facilitates their percolation through the profile [86]. The increase in IR_{1540} values in the reference areas with depth may be associated with a decrease in the C/N ratio, confirming the relative enrichment in N-containing structures and suggesting a progressive degree of humification. The opposite occurs in vineyard areas, suggesting a lower proportion of nitrogenous compounds with depth [33]. Higher surface values of IR_{1080} were observed in the reference areas. The greater abundance of these groups, which have higher biochemical lability in the SOM composition of the surface horizon, is related to the contribution of plant biomass [33].

4.2. Cu, Zn, and Mn Contents in the Chemical Fractions of SOM

The higher Cu contents in the chemical fractions of SOM in the older vineyards of the Serra Gaúcha and Campanha Gaúcha regions can be attributed to the amounts of this metal added through fungicide applications, mainly Bordeaux mixture, over years of cultivation. Several studies have demonstrated the high affinity of this metal for SOM functional groups, leading to its rapid adsorption by this fraction once it reaches the soil [15,39].

The lower Cu_{HA} contents observed in the reference areas of both studied regions and the consequent increase in the vineyard areas suggest that Cu levels in the reference areas are derived from the parent material, whereas in vineyard areas, the increase points to the addition of this element in cationic form [87], i.e., via copper-based fungicide applications.

Unlike Zn and Mn, Cu was also distributed in the HA and FA fractions. Cu exhibits a strong interaction with SOM, largely due to its electronic configuration $[Ar]3d^{10}\,4s^{1}$ and its high reactivity with S- and N-containing groups, carboxylic groups, and phenolic groups of SOM [39,88–90]. This behavior results in high binding energy and low desorption rates [91]. In contrast, Zn has the electronic configuration [Ar]$3d^{10}$ $4s^2$, and Mn, [Ar]$3d^5$ $4s^2$.

For Zn, the non-detection of this element in the FA and HA fractions in both studied regions reinforces the concept of its low affinity for the functional groups present in SOM, especially when compared to Cu. A possible explanation for this is that Zn may be present in the studied soils in insoluble forms. About 90% or more of Zn in soils is found in insoluble forms, making it scarcely available in the soil solution [92]. These forms are typically associated with carbonates, oxides, or even phosphates [93,94]. Another important point is that during the extraction process of the alkaline extract used to obtain FA and HA, NaOH was used as the extractant. As a result, Zn tends to react with NaOH, forming zinc hydroxide [$Zn(OH)_2$], which precipitates and becomes insoluble, thus justifying the non-detection of this element in the obtained extracts.

As a result, it was observed that most or all the Zn was found in the Zn_{Hu} fraction. This may indicate the importance of Hu in Zn retention, highlighting the influence of this fraction on the retention of this element, given its high affinity for soil mineral groups [95]. However, it is important to emphasize that the methodology used for obtaining Zn bound to Hu also promoted the release of Zn from both the carbon fraction and from sites adsorbed onto clay mineral groups. Since Zn has a high affinity for soil mineral groups, the final step of the fractionation process may have favored its solubilization from the residual soil [53].

According to the hard and soft acids and bases theory, Zn is classified as a soft acid [96], which may explain its greater affinity for a less acidic SOM fraction, i.e., the Hu fraction. Like Cu, Zn_{HCl} showed higher proportions in the surface layers of vineyards with longer cultivation periods, as successive applications of this element via fungicides increase the amount of Zn retained in lower-energy adsorption sites, thus facilitating its extraction with HCl.

Regarding the distribution of Mn in SOM fractions, its mobility in the soil depends on several factors, such as acidity, moisture, organic matter content, biological activity, among others [97,98]. In general, Mn mobility increases under low pH or low redox potential conditions. When soil pH exceeds 6, as observed in the soils of the study regions (Table A1), Mn tends to bind to organic matter, oxides, and silicates, thereby reducing its solubility.

In soils with higher pH levels, such as those observed in this study (Table A1), there is an increase in OH^- concentration in the soil solution, which promotes the deprotonation of H^+ ions from the surface of functional groups. This process increases the soil's CEC and enhances metal adsorption, thereby reducing metal concentrations in the soil solution and, consequently, the potential for toxicity [23].

This excess of OH^- ions in the soil solution can react with metals, forming Mn hydroxides, such as manganate [99], for example, which precipitate and reduce the activity of this element [23]. This may be a possible explanation for the increase in Mn levels in the MnHu fraction in both studied regions.

Mn tends to accumulate in the fractions associated with clay minerals and the residual fraction [38]. Thus, a similar process to that observed for Zn may have occurred. The HCl extraction likely removed the more weakly adsorbed Mn, while those with higher binding energy remained, resulting in higher percentages of Mn associated with the Hu. However, a small proportion of Mn was also found in the HA. This highlights the importance of this fraction in binding with this metal, despite its smaller proportion relative to the other SOM fractions (Figure 1), thereby reducing Mn availability to grapevine plants.

5. Conclusions

Land use changes affected the forms and distribution of soil SOC and metals, consequently altering soil quality. Elemental and spectroscopic analyses revealed that HA in native areas exhibited a more aliphatic character and higher polysaccharide content, indicating less stabilized organic fractions. In contrast, HA in vineyard soils were more humified and aromatic, particularly in surface layers, as confirmed by higher C_{HA}/C_{FA} and $(C_{HA}+C_{FA})/C_{Hu}$ ratios

Although SOC levels initially declined after land use change, long-term vineyard cultivation—especially under minimal soil disturbance—led to gradual SOC accumulation, approaching levels found in native areas. The use of cover crops further enhanced SOC content and contributed to conservation-oriented soil management.

Regarding metal distribution, Cu showed high affinity for HA and FA, reducing its bioavailability through complexation with SOM functional groups. In contrast, Zn and Mn were predominantly associated with the Hu, likely due to stronger interactions with soil clay minerals. These findings highlight the central role of SOM in regulating metal retention and improving soil quality in vineyard agroecosystems.

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Data Availability Statement: The datasets generate during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Appendix A

Table A1. Physical and chemical characterization of the 0.00-0.10 m and 0.10-0.20 m layers of soils from reference areas and vineyards with different cultivation times in Serra Gaúcha and Campanha Gaúcha.

Area	Layer (m)	Clay	TOC	pH_{H2O}	SMP Index	Cu	Zn	Mn	P	K	Ca	Mg	Al	H+Al	$CEC_{ef.}$	$CEC_{pH7.0}$	m	V
		----- g kg^{-1} -----				----------------------- mg kg^{-1} ---------- -----------					-------------------------- $cmol_c$ kg^{-1} ----------- --------------						----- % -----	
								Serra Gaúcha										
F	0.00-0.10	261	62.27	6.32	6.75	4.38	2.34	13.12	12.46	215.33	3.74	0.99	0.00	1.77	5.19	6.96	0.00	74.49
	0.10-0.20		24.72	6.20	6.77	0.27	2.06	27.09	4.40	48.00	2.62	0.37	0.00	2.05	3.05	5.10	0.00	59.42
V35	0.00-0.10	182	51.64	6.94	7.24	5.70	0.41	12.56	232.47	339.50	2.99	1.02	0.00	1.11	4.79	5.89	0.00	81.15
	0.10-0.20		28.49	6.76	6.83	1.34	0.84	6.35	79.20	264.83	2.32	1.16	0.00	1.71	4.23	5.93	0.00	70.92
V37	0.00-0.10	342	73.20	7.00	7.16	5.16	0.68	13.30	212.11	233.17	4.87	1.42	0.00	1.23	6.94	8.17	0.00	84.65
	0.10-0.20		23.83	6.87	6.87	1.94	0.48	6.31	13.19	180.33	3.00	1.41	0.00	1.68	4.80	6.48	0.00	74.01
V39	0.00-0.10	301	29.24	6.23	6.73	3.96	1.94	8.05	26.95	176.84	1.62	1.09	0.00	1.95	1.75	3.70	0.00	40.22
	0.10-0.20		13.97	6.17	6.92	1.35	0.12	4.06	14.11	109.34	1.33	1.08	0.00	1.54	1.46	2.99	0.00	39.71
								Campanha Gaúcha										

NG	0.00-0.10	74	25.55	5.57	6.71	0.14	0.90	47.09	6.69	36.09	0.61	1.05	0.13	1.95	1.88	3.70	7.35	47.10
	0.10-0.20		18.28	5.02	6.47	0.16	1.05	67.90	4.19	25.50	0.27	0.33	0.50	2.55	1.17	3.23	42.69	20.86
V13	0.00-0.10	80	30.75	6.30	6.86	0.16	1.51	10.46	66.47	47.84	3.16	4.60	0.03	1.67	7.90	9.54	0.68	78.24
	0.10-0.20		16.79	5.70	6.64	0.22	3.00	15.22	45.81	37.67	1.34	1.73	0.10	2.14	3.27	5.30	3.14	59.93
V19	0.00-0.10	74	22.84	6.33	6.86	1.11	2.00	9.28	89.51	45.25	1.91	1.49	0.03	1.70	3.55	5.21	1.01	67.95
	0.10-0.20		15.72	6.07	6.88	2.09	3.88	18.79	69.15	27.33	1.22	1.16	0.05	1.65	2.50	4.10	2.16	60.30
V36	0.00-0.10	54	23.71	6.24	6.89	7.10	1.34	11.63	60.45	48.29	2.57	4.36	0.07	1.61	7.11	8.66	2.79	77.34
	0.10-0.20		14.08	5.96	6.89	13.85	20.49	37.81	30.97	28.84	1.29	1.86	0.06	1.64	3.27	4.85	1.98	66.27

F: forest; V35: Vineyard with 35 years of cultivation; V37: Vineyard with 37 years of cultivation; V39: Vineyard with 39 years of cultivation; NG: Native grassland; V13: Vineyard with 13 years of cultivation; V19: Vineyard with 19 years of cultivation; V36: Vineyard with 36 years of cultivation; contents of total organic carbon (TOC); pH$_{H2O}$ (1:1 v/v); available contents of P, K^{+}, Cu^{+2}, Zn^{+2}, and Mn^{+2} extracted with Mehlich-1 extractor; exchangeable contents of Al^{+3}, Ca^{+2}, and Mg^{+2} extracted by 1 mol L^{-1} KCl [41]; With the obtained data, the potential cation exchange capacity (CEC$_{pH7.0}$) and effective (CEC$_{ef.}$), saturation by Ca+Mg+K+Na and by Al were calculated [42].

Appendix B

Table A2. Cu Contents in SOM Fractions in Vineyards with Different Cultivation Periods in the Serra Gaúcha and Campanha Gaúcha Regions.

Layer (m)	Serra Gaúcha					CV, %	Layer (m)	Campanha Gaúcha					CV, %
	F	V35	V37	V39	V39IR			NG	V13	V19	V36	V36IR	
	mg kg^{-1}							mg kg^{-1}					
		Cu_{HCl}											
0.00 – 0.05	0.53 aA	0.48 aA	0.55 aA	0.34 bB	0.31 aB	9.70	0.00 – 0.05	0.14 aB	0.12 ^{ns}B	0.14 aB	0.13 ^{ns}B	0.17 aA	9.57
0.05 - 0.10	0.28 bA	0.19 bB	0.31 bA	0.32 bA	0.16 bB	17.07	0.05 - 0.10	0.12 bA	0.09 B	0.09 bB	0.09 B	0.11 bA	6.54
0.10 - 0.15	0.28 bB	0.18 bCD	0.20 bC	0.43 aA	0.16 bD	6.40	0.10 - 0.20	0.12 b^{NS}	0.10	0.07 a	0.12	0.10 b	28.87
0.15 - 0.20	0.19 cB	0.13 bCD	0.18 bBC	0.27 bA	0.11 bD	17.76	0.20 - 0.40	0.10 cAB	0.11 AB	0.08 aB	0.10 AB	0.11 bA	16.95
CV, %	9.19	10.40	18.42	7.94	13.57		CV, %	3.77	18.61	14.13	29.29	7.70	
		Cu_{FA}											
0.00 - 0.05	16,5 abC	54,4 aA	47,3 aAB	20,3 bBC	53,9 aA	41,91	0.00 – 0.05	1,8 ^{ns}E	4,4 aD	10,2 abB	7,6 aC	12,9 aA	14,18
0.05 - 0.10	20,9 aC	28,2 bB	6,6 cD	27,5 aB	39,2 abA	10,09	0.05 - 0.10	1,6 B	4,2 aB	12,1 aA	5,3 bB	4,9 bB	41,47
0.10 - 0.15	10,2 bB	27,9 bA	26,7 bA	10,1 cB	11,6 abB	14,47	0.10 - 0.20	1,4 B	2,0 bB	4,4 bcA	4,7 bA	4,8 bA	12,63
0.15 - 0.20	11,2 bB	17,4 cA	23,5 bA	10,5 cBC	6,2 bC	17,89	0.20 - 0.40	1,8 B	1,8 aB	1,5 cB	2,8 cA	3,5 bA	9,89
CV, %	17,87	10,31	9,18	14,28	64,37		CV, %	20,07	13,98	38,29	12,81	10,67	
		Cu_{HA}											
0.00 - 0.05	6.2 cD	53.6 aC	122.9 aA	17.0 cD	71.4 aB	13.63	0.00 – 0.05	0.9 ^{ns}C	17.4 aB	50.7 aA	44.9 aA	44.6 aA	24.82
0.05 - 0.10	30.8 aB	21.4 bBC	25.0 bB	15.2 cC	45.0 bA	18.99	0.05 - 0.10	0.,8 D	7.7 bC	11.4 bBC	13.3 bB	19.5 bA	21.56
0.10 - 0.15	14.1 bcC	13.5 bC	29.9 bAB	31.8 aA	22.2 cBC	22.19	0.10 - 0.20	1.0 C	4.0 cB	5.1 bA	5.5 bA	6.0 cA	12.11
0.15 - 0.20	18.9 bC	11.3 bD	27.3 bA	24.8 bAB	21.4 cBC	10.15	0.20 - 0.40	0.7 D	3.4 cBC	2.4 bC	4.3 bAB	4.9 cA	23.95
CV, %	17.43	31.94	10.80	5.59	14.47		CV, %	43.47	12.09	33.71	40.55	6.86	
		Cu_{Hu}											

0.00 - 0.05	84.1 aE	31.9 cD	352.7 bC	1127.1 aB	1534.3 aA	4.13	0.00 – 0.05	2.4 bB	3.3 bB	47.5 aA	44.1 aA	18.6 aB	48.33
0.05 - 0.10	13.2 bE	214.6 abD	499.4 aC	574.3 cB	1110.6 bA	5.40	0.05 - 0.10	2.1 bD	1.0 bD	20.6 abB	37.3 bA	6.2 bC	15.26
0.10 - 0.15	70.1 aD	239.2 aC	454.5 aB	939.7 bA	438.0 cB	4.21	0.10 - 0.20	12.4 aA	1.3 bC	8.2 bB	9.4 cAB	3.0 bC	26.29
0.15 - 0.20	81.9 aE	201.4 bD	331.4 bB	372.7 dA	249.1 dC	8.36	0.20 - 0.40	2.6 bC	61.8 aA	4.8 bB	5.2 cB	2.5 bC	7.05
CV, %	8.60	8.09	5.49	3.58	4,.1		CV, %	17.34	11.49	61.50	8.87	23.37	
		Cu_{Total}											
0.00 - 0.05	107.3 abE	140.4 cD	523.4 abC	1164.8 aB	1660.0 aA	1.95	0.00 – 0.05	5.2 bD	25.3 bC	108.5 aA	96.9 aA	76.3 aB	14.5
0.05 - 0.10	62.0 cD	264.4 aC	561.5 aB	617.4 bcA	1195.0 bA	4.49	0.05 - 0.10	4.7 bE	13.0 bD	36.7 bB	55.9 bA	30.7 bC	7.56
0.10 - 0.15	94.5 bD	280.7 aC	421.6 abB	864.0 bA	472.1 cB	20.77	0.10 - 0.20	14.9 aBC	7.4 bD	28.6 bAB	19.7 cA	13.9 cC	11.95
0.15 - 0.20	112.2 aD	230.2 bC	330.0 bA	408.3 cA	276.9 dB	7.16	0.20 - 0.40	5.3 bD	67.1 aA	13.9 bC	12.3 cB	11.0 cBC	6.16
CV, %	5.44	5.29	18.57	13.72	2.50		CV, %	10.74	5.97	40.48	12.54	4.49	

F: Forest; V35: Vineyard with 35 years of cultivation; V37: Vineyard with 37 years of cultivation; V39: Vineyard with 39 years of cultivation; V39IR: Vineyard with 39 years of cultivation, with soil collected between the planting lines; NG: Native grassland; V13: Vineyard with 13 years of cultivation; V19: Vineyard with 19 years of cultivation; V36: Vineyard with 36 years of cultivation; V36IR: Vineyard with 36 years of cultivation, with soil collected between the planting lines; Cu_{HCl}: Copper present in non-humic substances; Cu_{FA}: Copper present in fulvic acids; Cu_{HA}: Copper present in humic acids; Cu_{Hu}: Copper present in humin; Cu_{Total}: Sum of copper contents from all fractions. CV: coefficient of variation. Means followed by the same lowercase letter in the column do not differ from each other by the Tukey test ($p < 0.05$). Means followed by the same uppercase letter in the row and within each region do not differ from each other by the T test (LSD) ($p < 0.05$).

Appendix C

Table A3. Zn Contents in the Chemical Fractions of SOM in Vineyard Soils with Different Cultivation Periods in the Serra Gaúcha and Campanha Gaúcha Regions.

Layer (m)	Serra Gaúcha F $mg\ kg^{-1}$	V35	V37	V39	V39IR	CV, %	Layer (m)	Campanha Gaúcha NG $mg\ kg^{-1}$	V13	V19	V36	V36IR	CV, %
		$Zn_{HC.}$											
0.00 – 0.05	15.16 bC	40.3 aB	42.5 bcB	36.8 cB	333.1 aA	7,.5	0.00 – 0.05	0.75 aB	13.20 aA	0.00 aB	0.00 aB	0.00 aB	49.85
0.05 – 0.10	8.06 cC	42.7 aBC	19.5 cC	76.6 bB	239.5 bA	33.46	0.05 - 0.10	0.27 aB	1.41 bA	0.00 aC	0.00 aC	0.00 aC	19.82
0.10 – 0.15	5.04 cC	3.18 bC	123.3 aA	106.5 aB	127.9 cA	9.52	0.10 - 0.20	0.19 aA	0.22 bA	0.00 aB	0.00 aB	0.00 aB	64.89
0.15 – 0.20	44.6 aAB	2.88 bB	84.3 abA	56.9 bcAB	60.7 dAB	7.58	0.20 - 0.40	0.19 aA	0.16 bA	0.00 aB	0.00 aB	0.00 aB	79.36
CV, %	22.07	12.88	30.93	11.84	3.99		CV, %	65.96	41.12	0.00	0.00	0.00	
		Zn_{Hu}											
0.00 – 0.05	163.4 aBC	174.4 ^{ns}B	138.6 bC	336.9 aA	36.5 cD	11.24	0.00 – 0.05	4.89 bD	6.82 bCD	13.08 abC	50.70 aA	26.18 aB	19.93
0.05 – 0.10	102.1 bB	227.9 A	244.4 aA	197.3 bA	47.8 cC	17.71	0.05 - 0.10	4.30 bC	12.20 bB	17.40 aA	14.37 bAB	16.75 bAB	21.38
0.10 – 0.15	153.7 aB	244.4 A	139.2 bBC	160.5 bB	104.3 bC	13.37	0.10 - 0.20	16.16 aA	11.53 bB	6.38 abC	6.30 cC	11.03 cC	18.03
0.15 – 0.20	116.5 c^{NS}	204.9	144.1 ab	184.7 b	156.1 a	31.52	0.20 - 0.40	5.76 bB	35.68 aA	5.63 bB	8.70 bcB	6.68 cB	52.05
CV, %	13.31	15.55	23.46	12.24	17.56		CV, %	22.56	47.57	41.30	13.02 bc	11.53	
		Zn_{Total}											
0.00 – 0.05	178.5 aB	214.7 bB	181.1 bB	373.8 aA	369.6 aA	8.62	0.00 – 0.05	5.65 bD	20.03 abB	13.08 ^{ns}C	50.70 aA	26.18 aB	16.10
0.05 – 0.10	110.2 bC	270.7 aB	263.9 aB	273.9 bAB	287.8 bA	3.30	0.05 - 0.10	4.57 bB	13.62 bA	17.4 A	14.37 bA	16.75 bA	20.82
0.10 – 0.15	158.7 aB	247.6 aA	243.7 aA	266.9 bA	232.2 cA	9.24	0.10 - 0.20	16.35 aA	11.75 bB	7.90 C	6.30 cC	11.03 cC	18.15
0.15 – 0.20	161.1 aB	207.9 bA	225.8 abA	241.7 bA	216.8 cA	9.82	0.20 - 0.40	5.95 bB	35.85 aA	10.63 B	8.70 bcB	6.68 cB	51.81
CV, %	5.48	4.92	9.79	9.28	6.66		CV, %	22.58	37.73	52.07	13.02	11.53	

F: Forest; V35: Vineyard with 35 years of cultivation; V37: Vineyard with 37 years of cultivation; V39: Vineyard with 39 years of cultivation; V39IR: Vineyard with 39 years of cultivation, with soil collected between the planting lines; NG: Native grassland; V13: Vineyard with 13 years of cultivation; V19: Vineyard with 19 years of cultivation; V36: Vineyard with 36 years of cultivation; V36IR: Vineyard with 36 years of cultivation, with soil collected between the planting lines; Zn_{HCl}: Zinc present in non-humic substances; Zn_{Hu}: Zinc present in humin; Zn_{Total}: Sum of zinc contents from all fractions. CV: coefficient of variation. Means followed by the same lowercase letter in the column do not differ from each other by the Tukey test ($p < 0.05$). Means followed by the same uppercase letter in the row and within each region do not differ from each other by the T test (LSD) ($p < 0.05$).

Appendix D

Table A4. Mn Contents in the Chemical Fractions of SOM in Vineyard Soils with Different Cultivation Periods in the Serra Gaúcha and Campanha Gaúcha Regions.

			Serra Gaúcha						Campanha Gaúcha				
Layer (m)	F mg kg^{-1}	V35	V37	V39	V39IR	CV, %	Layer (m)	NG mg kg^{-1}	V13	V19	V36	V36IR	CV, %
		Mn_{HCl}											
0.00 – 0.05	424.4 aA	308.8 aC	349.3 aB	250.9 aD	237.5 aD	6.14	0.00 – 0.05	39.4 ^{ns}A	60.2 aA	41.7 aA	0.00 aB	0.00 aB	63.52
0.05 – 0.10	280.7 bA	79.8 bC	234.6 bB	208.6 bB	99.4 bC	9.26	0.05 – 0.10	32.7 A	14.4 bB	13.7 bB	0.00 aC	0.00 aC	60.16
0.10 – 0.15	244.9 bA	44.1 cD	148.6 cC	214.2 abB	54.4 cD	8.09	0.10 – 0.20	16.2 A	10.4 bcB	6.2 cC	0.00 aD	0.00 aD	34.39
0.15 – 0.20	123.6 cA	48.1 cB	115.9 cA	123.1 cA	24.5 cC	6.26	0.20 – 0.40	16.9 A	4.5 cB	2.3 cB	0.00 aB	0.00 aB	80.79
CV, %	7.10	7.74	6.19	8.01	11.35		CV, %	46.83	13.17	11.45	0.00	0.00	
		Mn_{FA}											
0.00 – 0.05	122.0 aA	37.6 aB	29.5 aB	6.5 bC	45.6 aB	24.94	0.00 – 0.05	-	-	-	-	-	-
0.05 – 0.10	80.6 bA	8.3 bBC	0.5 cC	17.0 aB	13.4 bB	21.97	0.05 – 0.10	-	-	-	-	-	-
0.10 – 0.15	11.5 cA	8.4 bB	11.2 bA	6.7 bC	5.42 bD	4.97	0.10 – 0.20	-	-	-	-	-	-
0.15 – 0.20	18.9 cA	8.2 bBC	9.27 bB	5.9 bBC	2.8 bC	33.81	0.20 – 0.40	-	-	-	-	-	-
CV, %	14.54	5.57	14.11	9.50	73.24		CV, %	-	-	-	-	-	
		Mn_{HA}											
0.00 – 0.05	137.8 aA	137.84 aA	134.2 aA	64.2 aB	123.0 aA	15.90	0.00 – 0.05	5.0 aC	9.2 aBC	13.5 aB	11.6 aB	21.4 aA	24.00
0.05 – 0.10	83.8 bA	41.1 bC	55.7 bB	57.3 bB	17.8 bD	14.65	0.05 – 0.10	3.8 aB	5.3 bA	4.7 bAB	3.3 bC	5.9 bA	18.34
0.10 – 0.15	55.2 cA	27.8 bB	47.0 bA	42.0 bA	12.2 bC	12.01	0.10 – 0.20	2.1 bB	3.4 cA	2.1 bB	1.9 bA	2.3 cB	11.91
0.15 – 0.20	48.6 cA	40.1 bB	44.2 bB	25.0 bC	7.1 bD	7.26	0.20 – 0.40	1.4 bC	1.6 dAB	0.8 bD	1.6 bAB	1.9 cA	17.05
CV, %	12.38	20.52	11.38	5.21	37.03		CV, %	17.77	5.09	58.52	17.38	12.99	
		Mn_{Hu}											
0.00 – 0.05	5157.7 aA	1805.9 bC	1080.2 bD	3346.8 ^{ns}B	3108.5 abB	11.28	0.00 – 0.05	127.3 ^{ns}B	215.4 aB	288.6 aA	263.7 aA	215.0 aAB	32.80
0.05 – 0.10	3237.7 bAB	2426.2 abC	2866.8 aBC	3628.8 A	35394.0 aA	7.78	0.05 – 0.10	113.2 C	129.9 bB	165.8 bA	165.7 bA	124.5 bC	15.81
0.10 – 0.15	5820.3 aA	2556.8 aD	3326.2 aBC	3447.8B	2867.2 bCD	8.01	0.10 – 0.20	98.7 B	123.8 bB	162.0 bA	126.5 cB	59.6 cB	11.85
0.15 – 0.20	5975.1 aA	2581.0 aC	3004.0 aC	4100.3 B	1909.3 cD	9.15	0.20 – 0.40	93.6 B	102.3 bA	108.2 bB	133.6 cB	119.2 bB	10.24
CV, %	7.28	11.39	10.70	9.77	6.44		CV, %	13.94	10.60	4.90	5.00	12.47	
		Mn_{Total}											
0.00 – 0.05	5841.9 bA	2290.1 ^{ns}C	1593.2 bD	3638.3 ^{ns}B	3514.5 aB	9.99	0.00 – 0.05	143.1 ^{ns}B	193.3 aB	343.7 aA	275.3 aAB	236.3 aAB	33.26
0.05 – 0.10	3682.7 aA	2555.5 C	3174.5 aB	3911.7 A	3669.9 aA	7.30	0.05 – 0.10	130.0 C	149.5 bBC	184.2 bA	167.6 bAB	65.5 cD	13.10
0.10 – 0.15	6125.3 aA	2637.1C	3094.9 aB	3710.7 B	2939.3 bC	7.72	0.10 – 0.20	145.6AB	116.1 bC	159.5 bA	135.3 cBC	126.8 bBC	11.70

0.15 – 0.20	6172.7 aA	2677.5 C	3008.5 aC	4254.3 B	1943.7 cD	8.70	0.20 – 0.40	131.5B	221.5 aA	148.0 bB	129.8 cB	121.1 bB	9.82
CV, %	7.06	10.18	14.14	9.18	5.98		CV, %	8.72	8.66	4.55	4.60	11.60	

F: Forest; V35: Vineyard with 35 years of cultivation; V37: Vineyard with 37 years of cultivation; V39: Vineyard with 39 years of cultivation; V39IR: Vineyard with 39 years of cultivation, with soil collected between the planting lines; NG: Native grassland; V13: Vineyard with 13 years of cultivation; V19: Vineyard with 19 years of cultivation; V36: Vineyard with 36 years of cultivation; V36IR: Vineyard with 36 years of cultivation, with soil collected between the planting lines; Mn_{HCl}: Manganese present in non-humic substances; Mn_{FA}: Manganese present in fulvic acids; Mn_{HA}: Manganese present in humic acids; Mn_{Hu}: Manganese present in humin; Mn_{Total}: Sum of manganese contents from all fractions. CV: coefficient of variation. Means followed by the same lowercase letter in the column do not differ from each other by the Tukey test ($p < 0.05$). Means followed by the same uppercase letter in the row and within each region do not differ from each other by the T test (LSD) ($p < 0.05$).

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