

Article

The State of Soil Organic Carbon in Vineyards as Affected by Soil Types and Fertilization Strategies (Tri Morave Region, Serbia)

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Abstract: Due to specific soil properties and management practices, soils in vineyards are sensitive to degradation. The aims of this study were to examine (i) the state of soil organic carbon (SOC) in vineyards compared to other agricultural land, (ii) the influence of different fertilization strategies and soil type on SOC content and (iii) the rate of SOC change over time and potential of deep tillage for SOC preservation in subsoil. The study was carried out at 16 representative vineyard locations of the Tri Morave region, which represents the largest vine growing region in Serbia. The analyzed area included 56 vineyard plots. Results showed that SOC stocks in the topsoil and subsoil were lower than the average for agricultural land in Serbia. The soil type was an important predictor of carbon storage in the topsoil. An adequate application of inorganic fertilizers or green manure combined with farmyard manure initially resulted in the highest SOC contents. Continuous application of inorganic fertilizer without organic amendments has led to a decrease of SOC in topsoil. High rates of SOC stock change in topsoil accompanied a rapid reduction of SOC in the earlier stage of cultivation. In all investigated subsoils, SOC increased, except for unfertilized vineyards. Deep tillage has the potential to preserve SOC in the deeper soil layer and prevent carbon loss from the topsoil. More attention should be paid to the soil conservation practices to meet environmental sustainability of viticulture.

Keywords: soil; soil organic carbon; viticulture; fertilization strategies



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1. Introduction

Soil is an unrenewable natural resource and plays a key role in terrestrial ecosystems. Soil organic matter (SOM) is an essential component of soils as it supports soil structure, fertility and a range of physical properties that positively affect water availability to plants [1,2]. Consequently, a decrease of SOM can lead to drastic impairment of the soil physical and chemical properties, with negative impacts on soil nutrient cycling mechanisms [3,4]. In the light of the climate change debate, SOM is furthermore seen as an important storage pool for carbon. Soil organic carbon (SOC) sequestration is regarded as an option to mitigate climate change. Against this background, extensive research efforts have been devoted to the study of the terrestrial carbon cycle [5–9].

The strategy of SOC sequestration is built on a positive SOC budget for specific land use and management systems, whereby the input of C into soils exceeds the losses of SOC through erosion, mineralization/volatilization and leaching [10]. The total SOC stock of planet Earth has been estimated to be around 1500 Pg in the upper 100 cm of soil (700 Pg in the upper 30 cm), approximately three times higher than in the vegetation and double than in the atmosphere (about 560 and 760 Pg, respectively) [11–16]. The estimation of the global SOC stock up to 2 m depth is 2060 ± 215 Pg [17]. These values represent only

a rough estimation, with errors estimated as between 504 and 3000 Pg [18]. Due to the high potential of soils for carbon sequestration, the “4 per 1000” international initiative was launched in Paris in 2015 [19] to increase the awareness of land use responsibility for climate change.

One of the main factors that control the vertical distribution of SOC is land use [14,20]. Changes in land use are the second most important source of GHG emissions to the atmosphere after fossil fuel burning [21]. According to Wiesmeier et al. [22], the main factors affecting the variation in SOC stocks in Bavaria (Germany) are land use and soil type. Other factors include farming/cropping systems, adoption of recommended management practices, tillage methods, use of organic and inorganic amendments etc. [10]. The factors responsible for the differences in SOC stocks can also differ with soil depth [23]. Hobley et al. [24] found that climatic factors have more effect at shallow depths, while site factors such as bulk density, soil type and parent rock became more important at depths below 20 cm.

In the vine-growing areas of the Republic of Serbia, frequent tillage between the rows is a common practice to keep the soil free of weeds. Such intensive working of vineyard soils can lead to soil degradation, with loss of soil fertility, acceleration of soil erosion and SOM mineralization, and CO₂ emission increase [25–27]. Due to specific soil properties in vineyards such as limited soil development, coarse texture and low capacity to protect SOM binding to soil minerals, these soils are sensitive to degradation [28–30] and lose potentially more SOM than other agricultural soils.

According to the assessment of the SOC stock in the vineyards of European countries, similar stocks were reported. The mean SOC stock in Italy in the top 30 cm of mineral soil of the vineyards was $41.9 \pm 15.9 \text{ t ha}^{-1}$ [31]. The SOC stocks in vineyards of France were $39.4 \pm 26.5 \text{ t ha}^{-1}$ [32]. In peninsular Spain, $42.5 \pm 28.9 \text{ t C ha}^{-1}$ was reported for vineyards [33].

Fertilization is one of the most important practices in crop production, and in intensive viticulture production in Serbia exclusively inorganic fertilizers are applied. Organic materials are rarely applied, only when establishing vineyards. Organic matter input from grapevine residues is limited, because Serbian winegrowers commonly do not bring grape pomace back to the soils. Some wine producers avoid using organic fertilizers for grape production, because they fear negative effects on the quality of the grapes. This practice potentially aggravates the decrease of SOC, which directly jeopardizes the long-term maintenance of soil quality. There is concern that if SOC content in vineyard soils is allowed to decrease beyond a critical threshold, the productive capacity of viticulture will be compromised by further deterioration in soil physical properties and by deterioration of soil nutrient cycling mechanisms [34]. However, to date there is no systematic monitoring of Serbian vineyard SOC contents available and long-term dynamics have also not been studied so far.

The aim of this study was to examine (i) the state of SOC in vineyards compared to other agricultural land, (ii) the rate of SOC change over time, (iii) the influence of different fertilization strategies and soil type on current SOC contents and (iv) the influence of deep tillage on SOC preservation in the deeper soil layer as a counterbalance against C losses in the topsoil.

2. Materials and Methods

2.1. Study Area

The study area is the vineyard region Tri Morave, Serbia (Figure 1), which represents the largest vine growing region in Serbia, with a surface area of 286,929 km². This region is located between 43°21' N and 44°07' S and includes the areas around three large rivers in Central Serbia, the wide lower basin of the Zapadna Morava River, the lower basin of the Južna Morava River and the wider upper basin of the Velika Morava River [35].

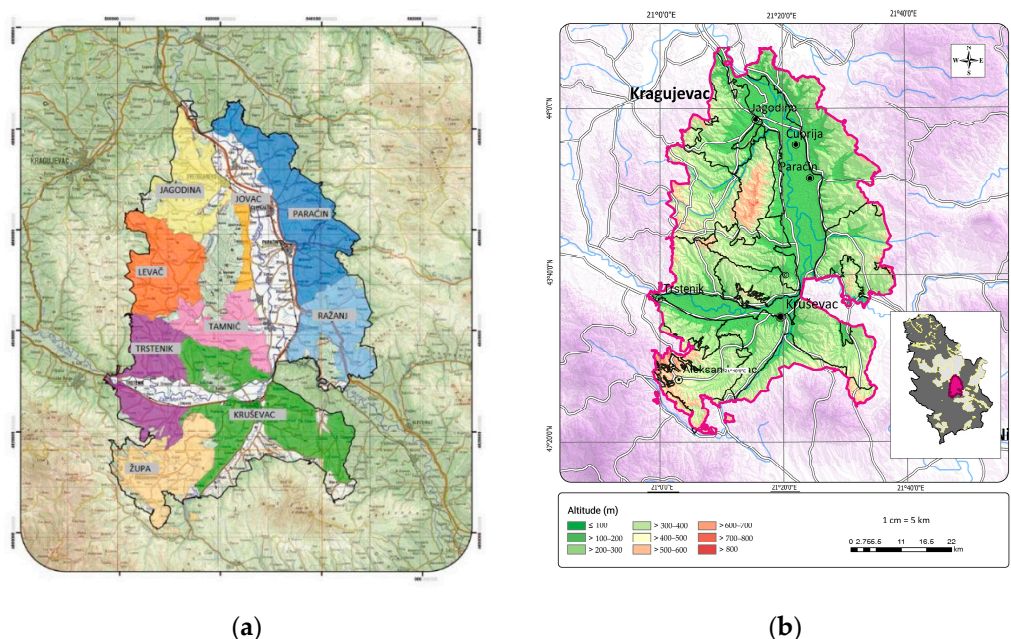


Figure 1. The vineyard region Tri Morave, Serbia: (a) vine growing districts (modified by authors) [36] and (b) topographic map.

Total areas of individual wine growing districts are much smaller than the areas of the subregions. Conversely, areas under vineyards are far smaller than the distribution of each wine growing district, as well as areas that have the potential for wine growing production. These areas have been increasing in recent years. Tri Morave vine region has 75.28 km² of vineyards from which 13.67 km² include table varieties and 61.61 km² include wine varieties [35].

Although it has a wide range of grape varieties, it is mostly well-known for its black grape varieties used in production of high quality red, but also rosé wines. The most widespread black varieties are Cabernet Sauvignon and Merlot. There are nine vine growing districts in this region: Paraćin, Jagodina, Jovac, Levač, Tamnić, Trstenik, Kruševac, Župa and Ražanj [36].

The Tri Morave wine region possesses favorable climatic conditions for vine growing. The average annual air temperature, for the last 50 years, is 11.4 °C and the average annual precipitation is 644 mm. The heat summation period (April–October), also known as the Winkler index, is 1571.5, placing the Tri Morave wine region in the II zone according to Winkler [36]. Most of the vineyards in the region are located on slopes (Figure 1b). The majority of them (49%) slope between 5 to 10 degrees, about 10% slope between 10 to 15 degrees and about 36% are located on flatter terrain [37].

There are three basic geomorphological units: alluvial plains of the Južna, Zapadna and Velika Morava rivers, dissected fluvial terraces in the zone of low hills and the mountain zone (Figure 2B). Since the parent substrate represents an essential determinant of geochemical, mineralogical and granulometric properties of soils, the Tri Morave wine region has a moderate pedological diversity dominated by different varieties of only two types of soil: cambisols and vertisols (Figure 2A) [37].

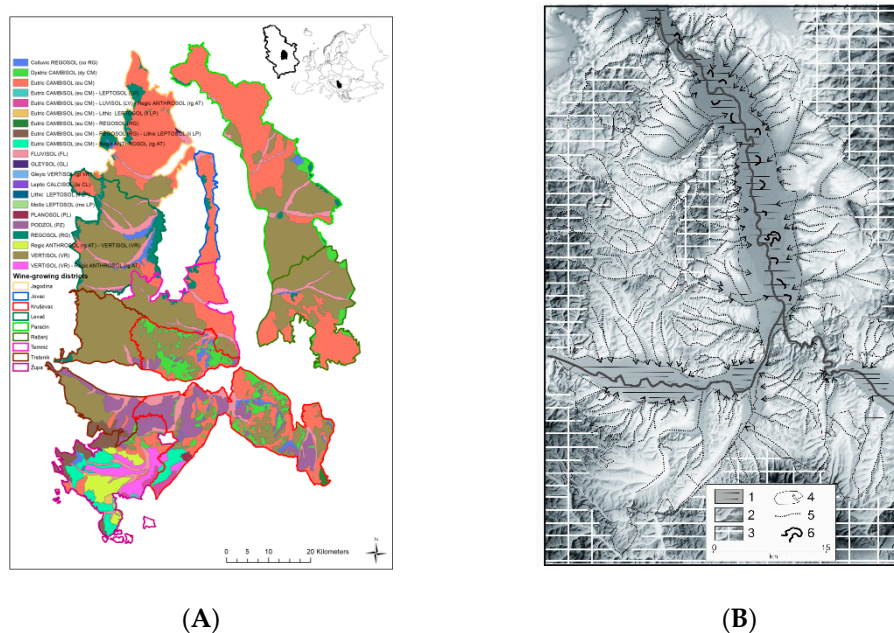


Figure 2. (A) Pedological map of the Tri Morava vineyard region and borders of vine growing districts, according the World Reference Base (WRB) soil classification, based on digitalized primary pedological map of the Republic of Serbia 1: 50,000. (B) Geomorphological map (1—alluvial plains, 2—dissected fluvial terraces in the zone of low hills, 3—mountain relief zone, 4—borders of the Tri Morava vineyard region, 5—a smaller river valley, 6—paleomeander).

2.2. Soil Sampling

The study was carried out in 16 representative vineyard locations (Figure 3 and Figure S1, and Table 1). The total analyzed area included 56 production vineyard plots. These plots exhibit a uniform microrelief and slope of the terrain, as well as having the same cultivation practices. The size of the plot (subplot) varied from 6000 to 40,000 m².

Table 1. Locations, vine growing districts and soil types of the vineyards.

No	Locality	Vine Growing District	Soil Type (FAO–WRB)
1	Levač	Levač	Haplic Vertisol (Clayic)
2	Dobričevo	Paraćin	Eutric Cambisol
3	Glavica	Levač	Haplic Vertisol (Clayic)
4	Oparić	Paraćin	Stagnic, Eutric Cambisol
5	Lozovik	Jagodina	Eutric Cambisol (Clayic)
6	Lučina	Kruševac	Eutric Cambisol (Clayic)
7	Ravnjak	Kruševac	Eutric Cambisol (Clayic)
8	Bučje	Trstenik	Skeletal Leptosol (Clayic)
9	Trstenik	Trstenik	Eutric Cambisol (Clayic)
10	Bačina	Temnić	Eutric Cambisol (Clayic)
11	Lipovac	Ražanj	Eutric Cambisol (Clayic)
12	Gornje Zleginje	Župa	Haplic Vertisol
13	Donje Zleginje	Župa	Haplic Vertisol
14	Tržac	Župa	Eutric Cambisol (Clayic)
15	Aleksandrovac	Župa	Haplic Vertisol
16	Aleksandrovac	Župa	Gleyic, Skeletal Fluvisol (Clayic)

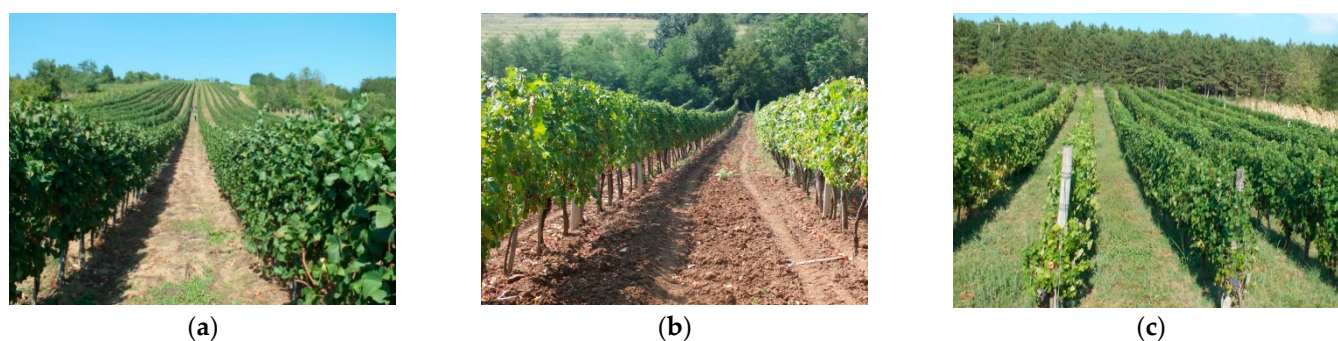


Figure 3. Some localities of observed vineyards: (a) Trstenik, (b) Bačina, (c) Glavica.

The soil was sampled from two depths, 0–30 and 30–60 cm, composing a mixed sample from 20 individual samples. The total number of the mixed soil samples was 112.

In order to determine the indigenous soil type of the vineyard, which was not altered by powerful ameliorative measures (deep tillage) during the vineyard establishment and turned into an Anthrosol (Eutric, Clayic, Regic), AT-eu.ce.rg, soil profiles were opened in a nondisturbed site of vineyards (Figure 4).

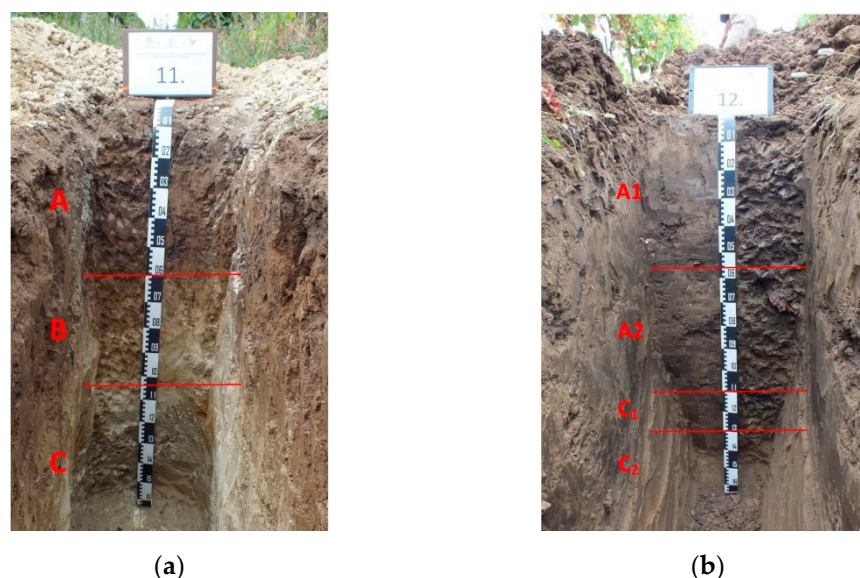


Figure 4. Soil profiles: (a) Locality Lipovac—Eutric Cambisol (Clayic), (CM-eu-ce). (b) Locality Gornje Zleginje—Haplic Vertisol, (VR-ha).

Soil profiles were analyzed at 16 representative locations, up to a maximum depth of 200 cm or to the parent material. Samples were taken in undisturbed (Kopecký cylinders, volume 100 cm³) and disturbed state using an Eijkelkamp percussion core sampler. The total number of this soil samples was 116. Field work took place during 2015.

Georeferencing of soil and parcel samples in this study was performed using GPS receivers (Trimble GPS GeoXH 3000, Trimble GPS Juno SC, Terrasync Professional software; Trimble, Inc., Sunnyvale, CA, USA). Data processing was carried out using the ESRI ArcEditor 10 GIS (geographic information system).

The initial SOC state of vineyards was obtained from the soil analysis before vineyards establishment, since 1975.

2.3. Fertilization Strategies

The fertilization strategies in the observed vineyards are given in Table 2. These data are derived from long-term management records for the period 1975–2015.

Table 2. The fertilization strategies in the observed vineyards.

AF + FM + NPK	Ameliorative fertilization initially + farmyard manure initially + continuous application of NPK ¹ inorganic fertilizers
AF + NPK	Ameliorative fertilization initially + continuous application of NPK ¹ inorganic fertilizers
AF + FM	Ameliorative fertilization initially + farmyard manure initially
FM + GM	Farmyard manure initially + green manure continuously
F	Foliar microbial fertilizer continuously
NF	No fertilizers

¹ N—nitrogen, K—potassium, P—phosphorus.

2.4. Laboratory Analysis

All laboratory analyses were performed at the Laboratory for Soil and Agroecology of the Institute of Field and Vegetable Crops, accredited according to the standard ISO/IEC 17025:2017 [38].

The soil samples collected were naturally air-dried, milled and passed through a 2.0 mm sieve, according to ISO 11464:2006 [39]. Soil pH value was determined by the potentiometric method according to ISO 10390:2005 [40] in a 1:5 suspension of soil in 1 M KCl using a Mettler Toledo SevenCompact pH meter with glass electrode (Mettler Toledo, LLC, Columbus, OH, USA). The carbonate content (as CaCO₃) was determined according to the ISO 10693:1995 [41] volumetric method. SOC was determined by elementary analysis using CHNSO VarioEL III Elementar (Elementar Analysensysteme GmbH, Langenselbold, Germany) after dry combustion and carbonate removal, in accordance with the ISO 10694:1995 [42]. The particle size distribution was determined in the <2 mm fraction using the pipette method [43]. The size fractions were defined as clay (<2 µm), silt (2–20 µm), fine sand (20–200 µm) and coarse sand (200–2000 µm).

Bulk density (BD) was determined from standard volume (Kopecký cylinders; 100 cm³) soil samples, which were dried to constant mass at 105 °C.

2.5. Calculation of SOC Stocks

Soil organic carbon stocks were calculated:

$$\text{SOC (t C ha}^{-1}\text{)} = \text{SOC (\%)} \times \text{BD (g cm}^{-3}\text{)} \times \text{SLT (cm)} \times [1 - (\text{GV}/100)]$$

where BD—bulk density; SLT—soil layer thickness; GV—gravel volume (%).

2.6. Statistical Analyses

Study data were processed by the methods of descriptive statistics. The effects of fertilizer strategies and soil type on SOC were estimated by an analysis of variance (ANOVA). The significances of the differences were determined using the Duncan's range test (multi-stage test) ($p < 0.05$). Regression analysis was used for estimating the relationships between soil depth and SOC content. Correlation analysis was calculated by using the Pearson correlation at a significance level of $p < 0.05$. All statistical analyses were performed using STATISTICA 12.6 (StatSoft, Inc. Corporation, Tulsa, OK, USA).

3. Results and Discussion

3.1. Characteristics of the Soil

Physical and chemical soil properties of the examined vineyards for the soil layers 0–30 cm, 30–60 cm and profile horizons (<200 cm) are given in Table 3. Soil pH value was highly acid to alkaline in topsoil and subsoil, according to classification for vineyard soils [44]. The topsoil layer (0–30 cm) has an acidic pH value for the most part (72% of the region's surface area). In the soil profile horizons, the pH value of most soils increased with

depth. The most suitable soil pH in terms of vine cultivation is neutral [45]. According to White [46] the optimum pH range for vine growth is 5.5–8. Slightly acidic and neutral vineyard soils generally have better nutrient balance for plant growth. Soil pH value is most often a natural property of the soil and comes from the pH reaction of the parent substrate in which the soil was formed.

Table 3. Descriptive statistics of soil properties in layers 0–30, 30–60 cm and profile horizons (<200 cm).

Soil Properties	Min.	Max.	Mean	Std. Dev.
0–30 cm				
pH (in 1M KCl)	3.94	7.60	5.53	1.14
CaCO ₃ (%)	0.00	18.45	1.29	2.87
Clay (%)	17.76	50.48	38.81	6.33
Silt (%)	16.36	32.36	25.48	3.70
Fine sand (%)	19.83	46.32	29.55	5.36
Coarse sand (%)	1.17	19.86	6.12	4.39
30–60 cm				
pH (in 1M KCl)	3.77	7.56	5.35	1.13
CaCO ₃ (%)	0.00	10.90	1.38	2.72
Clay (%)	13.96	49.96	39.13	6.65
Silt (%)	15.76	33.60	25.33	3.87
Fine sand (%)	20.34	47.62	29.49	5.46
Coarse sand (%)	1.16	17.66	6.05	4.40
profile horizons, 0–200 cm				
pH (in 1M KCl)	3.75	7.47	5.61	1.25
CaCO ₃ (%)	0.00	37.90	3.08	8.42
Clay (%)	15.84	54.16	40.96	8.24
Silt (%)	17.20	46.16	26.34	5.22
Fine sand (%)	18.91	40.71	27.12	5.50
Coarse sand (%)	1.09	20.47	5.59	4.67
Bulk density (g cm ⁻³)	1.24	1.70	1.52	0.13

Samples of topsoil and subsoil belong to the noncalcareous to highly calcareous soil category [44]. The content of CaCO₃ in completely carbonate-free soils is completely uniform in terms of profile depth or a small part of carbonates appears at the lower layer. In other soils, the carbonate content generally increases in depth of the profile. The content of free CaCO₃ largely depends on the parent substrate, i.e., the type of soil.

Bulk density (BD) of the soils varied between 1.24 and 1.70 g cm⁻³ (Figure 5). The BD of most of the examined soil profiles increases with depth, as a consequence of the long-term pressure of the upper soil layers on the lower layers. Most of the examined horizons have BD of more compact arable soils, according to the classification of Kačinski [47,48] which is unfavorable from the aspect of water, air and temperature regime of these soils. According to Leake [45] the BD values in the vineyard soils should be less than 1.4 g cm⁻³. In the study of Doğan and Gülser [49], BD of vineyards soils varied between 1.07 and 1.75 g cm⁻³.

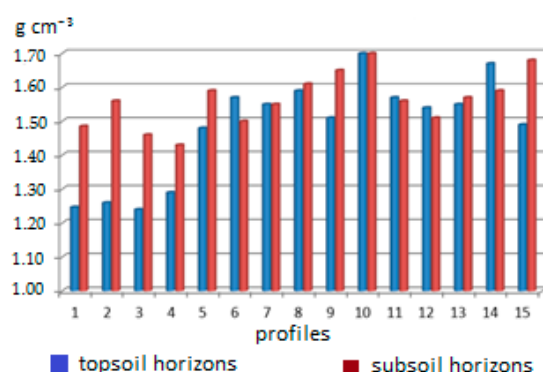


Figure 5. Bulk density of the soil profiles.

The soil texture examined for the Tri Morave wine growing region is characterized by increased clay content (Figure 6). Clay content of vineyard fields varies between 13.96% and 50.48%. Most of the samples are concentrated in the classes of light clay and heavy clay. This texture is unfavorable for most cultivated plant species. According to Gücüyen, loamy soils include high organic matter, low-water-holding capacity and well-drained characteristics that are generally suitable for good quality grape production [50].

3.2. Soil Organic Carbon Stock

The soil organic carbon stock in the organic layer (0–30 cm) of the observed vineyard soils ranged between 17.72 and 87.04 t ha⁻¹, with mean value 46.19 t ha⁻¹ (Figure 7). In subsoil (30–60 cm), SOC stock ranged between 14.54 and 91.16 t ha⁻¹, with mean value 40.26 t ha⁻¹. These results are lower than the average value for SOC stock of agricultural land in Serbia. The previous analysis of organic carbon content in agricultural land of Serbia showed that in the 0–30 cm layer, values of SOC stock ranged from 3.72 to 328.23 t ha⁻¹ with mean value 68.99 t ha⁻¹ [51].

Similar results were obtained by other authors. According to the assessment of the mean SOC stock of the different cropland uses in Italy, SOC stock in the top 30 cm of mineral soil for the vineyards was 41.9 ± 15.9 t ha⁻¹ [31]. In his study, SOC stock for the whole cropland category was 52.1 ± 17.4 t C ha⁻¹, which is in the range of those reported for other European countries. Smith et al. [52] suggest a mean value of 53 t C ha⁻¹ as an average value for all European cropland soils. SOC stocks in peninsular Spain showed a high heterogeneity, with the lowest values in arid regions. The average value in topsoil (0–30 cm) was 44 ± 26 and 57 ± 35 t C ha⁻¹ in subsoil (30–50 cm) [11]. SOC stock in vineyards of peninsular Spain was reported by Murillo [33] at 42.5 ± 28.9 t C ha⁻¹. For France, the SOC stock in the agricultural soils was estimated at 15 to 40 t ha⁻¹ in mid-France and 40–50 t ha⁻¹ in the north and southwest [53]. Results for SOC stocks in the vineyards of France were reported by Martin et al. [32] at 39.4 ± 26.5 t ha⁻¹.

Besides the specific soil properties in vineyards, the reduction of SOC is possibly a consequence of the intensification of agricultural practices [54,55]. In the observed area, the management is based on the reduced use of organic fertilizers, which are applied mainly only when establishing vineyards, as well as on conventional land cultivation in intensive production. Intensive viticulture could lead to the soil degradation, with loss of soil fertility, acceleration of soil erosion and SOM mineralization, and CO₂ emission increase [26,27,56]. Soil tillage affects soil respiration, temperature, water content, pH, oxidation–reduction potential and the soil ecology [57,58]. In particular, it enhances the microbial biomass turnover and, in turn, the short-term CO₂ evolution by improving soil aeration, increasing the contact between soil and crop residues and by exposing organic matter to microbial attack [59].

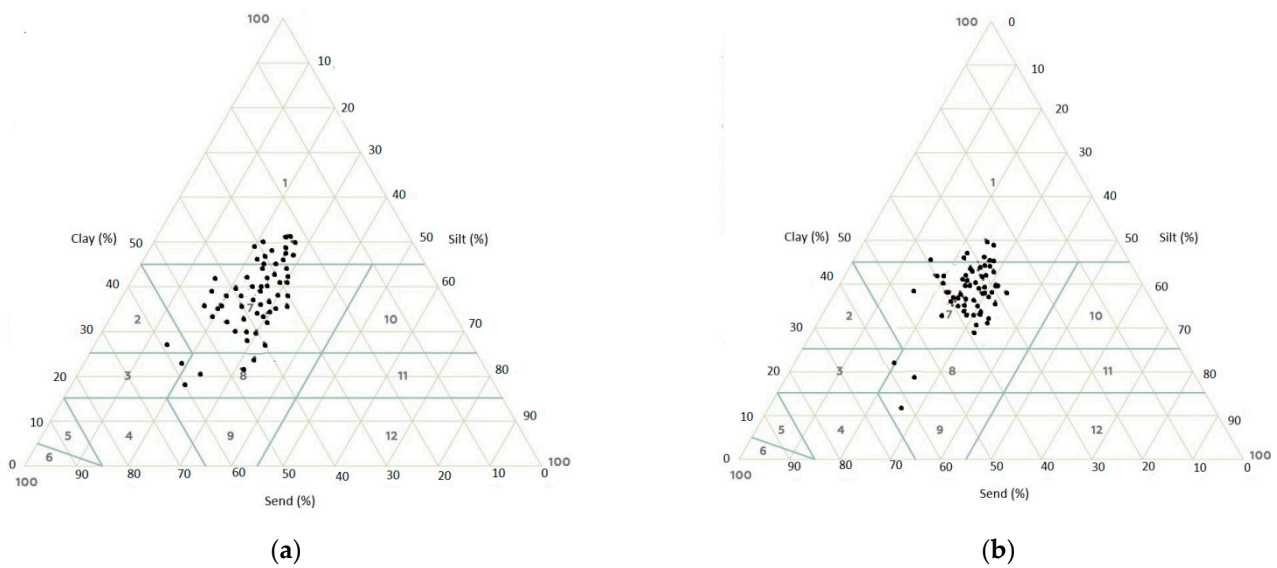


Figure 6. Textural triangle diagram according to International Union of Soil Sciences (IUSS) system of classification of soil particles: (a) soil depth 0–30 cm (N = 56) and (b) soil depth 30–60 cm (N = 56). Textural classes: 1—heavy clay, 2—sandy clay, 3—sandy clay loam, 4—sandy loam, 5—loamy sand, 6—sand, 7—light clay, 8—clay loam, 9—loam, 10—silty clay, 11—silty clay loam, 12—silt loam.

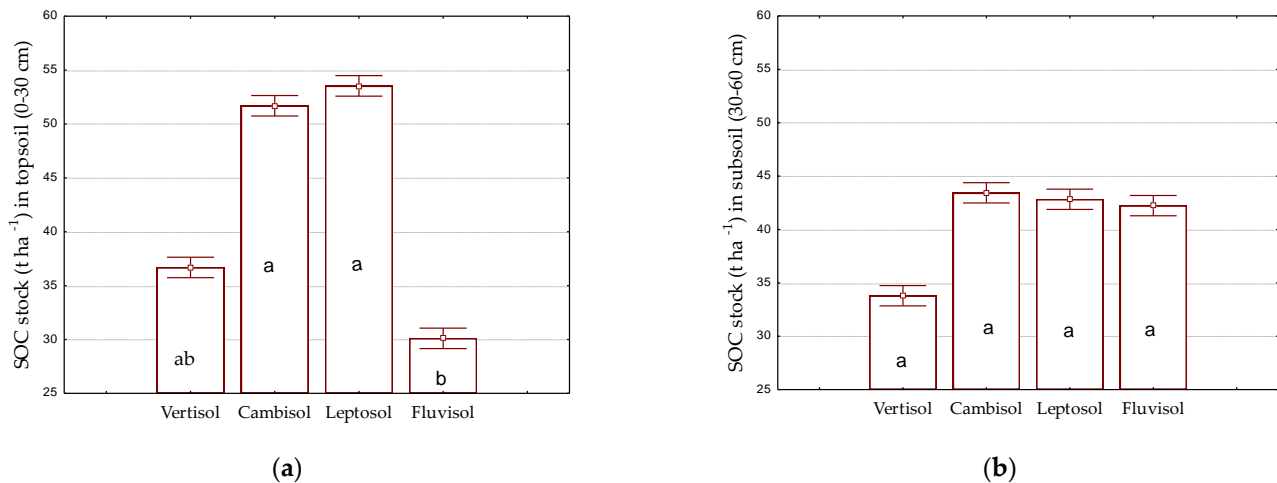


Figure 7. Soil organic carbon (SOC) stocks: (a) topsoil, 0–30 cm (N = 56), and (b) subsoil, 30–60 cm (N = 56). Significant differences among soil groups are labeled with different letters (Duncan's range test, $p < 0.05$).

Experiments conducted in the United States [60] show a reduction of more than 30% in the SOM content in soils that have been cultivated for many years. In the undisturbed state, SOM contents are the result of a balance between mineralization losses and organic matter inputs. Disturbances change this equilibrium very quickly, leading to a higher level of decomposition of SOM, especially the labile forms (sugars, amino acids) that play a major role in stabilizing the physical structures of the soil [51]. The remaining forms of SOM are less effective in stabilizing the soil structure. Such a system is in a state of degradation, which can be prevented by compensating for the loss of SOM by increasing organic matter input.

The results of our study confirmed that different soil types exhibited typical ranges topsoil carbon storage. Leptosols ($53.53\ t\ ha^{-1}$) yielded the highest SOC in topsoil. A similar value was observed in Cambisol ($51.69\ t\ ha^{-1}$). The comparison between SOC stocks of Fluvisols ($30.11\ t\ ha^{-1}$) and Vertisols ($36.69\ t\ ha^{-1}$) revealed no significant differences. A previous assessment of organic carbon stocks in the agricultural soils of the Republic of

Serbia [51] observed the following mean values for the reference soil groups: Leptosols (151.33 t ha⁻¹), Cambisols (89.81 t ha⁻¹), Vertisols (71.09 t ha⁻¹) and Fluvisols (70.80 t ha⁻¹). In this study, the mean values of SOC for observed soil type were higher than our results, but of the same order. In soils of the Vojvodina region, the largest SOC stocks were observed in Vertisols (74 t ha⁻¹) and the lowest Fluvisol (46 t ha⁻¹) [61]. Murillo [33] reported mean values of SOC stocks for peninsular Spain: 71.4 ± 57.8 t C ha⁻¹ for Cambisols; 75.8 ± 58.9 t C ha⁻¹ for Fluvisol; 98.8 ± 56.4 t C ha⁻¹ for Leptosol and 68.9 ± 37.8 t C ha⁻¹ for Vertisol. It may be concluded that SOC stocks in all of the observed soil types for vineyards were lower than the average for agricultural land in Serbia.

The highest SOC in subsoil were for Cambisols (43.43 t C ha⁻¹), Fluvisols (42.23 t C ha⁻¹) and Lepotsols (42.83 t C ha⁻¹) revealed similar SOC stocks in the soil horizon. The subsoil of Vertisols yielded the lowest amount of SOC (33.79 t ha⁻¹). The differences in SOC stocks of all soil groups in subsoils were not significant. Fluvisol contain much higher SOC stocks in subsoils than in topsoil. Schöning et al. [62] highlighted the importance of subsoil carbon balance on a plot scale. Grüneberg [63] showed that this is also true for the regional scale.

Differences of SOC stocks can be partly explained by soil texture, which is a result of different parent materials on which the soils developed. Cambisols are characterized by adequate profile depth, good texture and water–air properties. Fluvisols are formed due to the constant deposition of fresh suspensions and do not have a developed humus horizon. The humus content is low, about 2%, and often below 1%, and it is not distributed uniformly in depth. These characteristics can explain our results. The high concentration of SOC in Leptosols is a consequence of the humus layer in humus–carbonate and humus–silicate soils. Lower regions under natural vegetation contain 5–10% of humus, while higher ones can contain up to 20% [64]. Vertisols have low production value due to the high clay content and specificity of descending material from upper to lower layers due to the formation of cracks during the dry part of the year.

3.3. Organic Carbon Concentrations in Observed Soil Types

The mean SOC concentrations in the topsoil and subsoil of the observed soil types of the Tri Morave vineyard region are given in Table 4. The highest mean concentration of SOC in topsoil was found in Leptosols and Cambisols, and the lowest in Fluvisols. As for the SOC stocks, the organic carbon concentration in the agricultural soils was also lower in our results than in the previous assessment for the reference soil groups [51], in which the content ranged from 0.08% to 21.72%, with a mean value of 2.07% for the top 30 cm. In this study, Vertisols (1.76%) and Fluvisols (1.74%) were characterized as soils of low SOC concentration, while Leptosols (3.96%) and Cambisols (2.16%) belonged to the class with medium SOC contents.

Table 4. SOC concentrations (%) in soil layers at 0–30 and 30–60 cm (N = 112).

Soil Type	Min.	Max.	Mean	Std. Dev.
Eutric Cambisol	0.64	2.02	1.12	0.32
Haplic Vertisol	0.38	1.19	0.85	0.32
Skeletal Leptosol	0.94	1.25	1.12	0.16
Gleyic, Skeletal Fluvisol	0.62	0.73	0.67	0.07
30–60 cm				
Eutric Cambisol	0.31	1.15	0.93	0.32
Haplic Vertisol	0.38	1.03	0.74	0.32
Skeletal Leptosol	0.74	1.00	0.89	0.13
Gleyic, Skeletal Fluvisol	0.52	1.14	0.83	0.43

In the examination of SOC concentration of European soils, for all soil categories (arable, forest, grass and others) the following values were obtained: Cambisols 2.4%,

Fluvisols 1.6% and Vertisols 1.5% [16]. In the experiment of Novara et al. [25], which was carried out on a flat vineyard area in the west of Sicily, Italy, on calcic–gleyic vertisol, SOC content was $0.95 \pm 0.07\%$, similar to our results.

The overall mean SOC concentration of the samples in topsoil (0–30 cm), $1.02 \pm 0.32\%$, was higher than the SOC concentrations in subsoil (30–60 cm), $0.85 \pm 0.32\%$.

In the deeper layers, the SOC concentration was fairly uniform. The highest SOC content was also recorded in Cambisols, while the lowest was found in Vertisols.

Numerous studies reported a dominant effect of soil type on SOC stocks both in topsoil and subsoil [22,24,65]. Soil type is strongly associated with SOC storage at multiple scales and under different climatic conditions [66]. Soil type is not an independent control factor but integrates climate, parent material and topography related properties, which affect the potential of soils to store C, particularly through moisture regime and texture [66].

3.4. Distribution of Organic Carbon in the Soil Profile

The SOC content in soil profile horizons ranged from 0.09% to 1.79% (Figure 8). The highest SOC content was observed in the topsoil layer, as expected. This is a consequence of the accumulation of organic matter originating from plant residues, as well as higher activity of microorganisms, which participate in the decomposition of fresh organic matter. Significant factors in these processes are soil temperature and humidity.

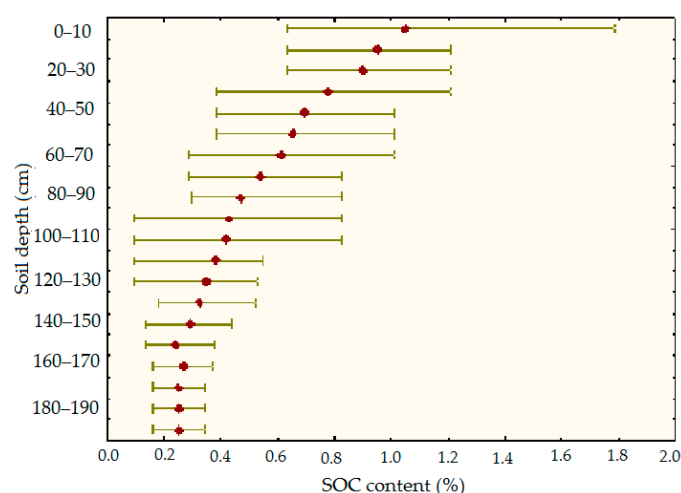


Figure 8. Vertical distribution of soil organic carbon (SOC) content in soil profile of vineyards, <200 cm (N = 60).

The average value of SOC decreased rapidly with increasing depth, which is in agreement with the results of other research [67–69]. Regression analysis revealed a statistically significant change in SOC content with soil depth (Figure 9). The average SOC content decreased by 0.62% in the 0–100 cm layer with increasing depth. The declining trend of SOC content decreases in the deeper layer, 100–200 cm, with the average SOC value falling by 0.17%.

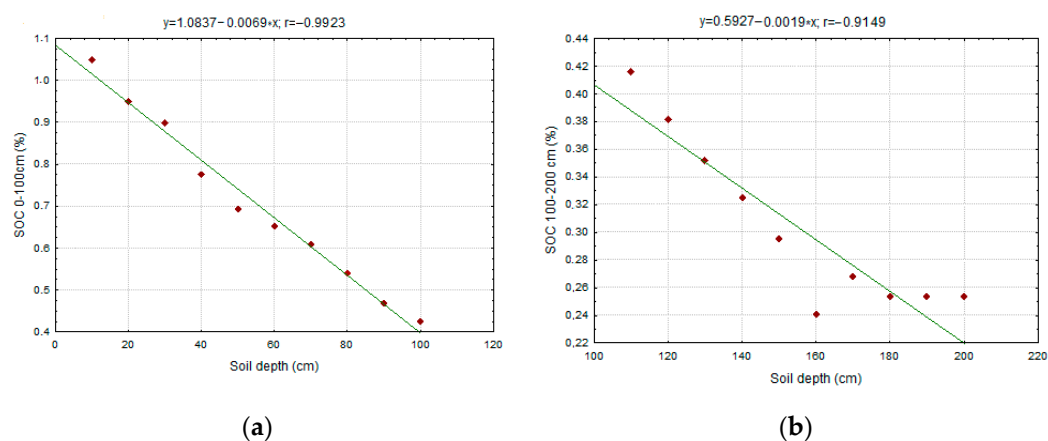


Figure 9. Regression analysis for the relationships between soil depth and SOC content in the soil profile of vineyards (<200 cm): (a) soil depth 0–100 cm and (b) soil depth 100–200 cm.

In the study by Yu et al. [67], the mean value of SOC in the 0–100 cm soil layer decreased rapidly with increasing soil depth, ranging from $3.37 \pm 1.43 \text{ g kg}^{-1}$ in the topsoil layer (0–20 cm) to $1.66 \pm 0.98 \text{ g kg}^{-1}$ in the 80–100 cm layer.

Correlation analysis showed a more significant correlation between SOC and soil depth in the upper layer ($r = 0.99$) compared to the deeper soil layer ($r = 0.91$).

3.5. Effect of Fertilization Strategies on SOC Concentration

Fertilizer management is important for increasing crop productivity and soil quality, while limiting the environmental contamination. In intensive vineyard production of Serbia, fertilization is mostly based on inorganic fertilizers. Some winegrowers avoid using organic amendments, because they fear negative effects on the quality of the grapes (extended period of grape ripening, low sugar and high acid content). However, several studies confirmed that the combined application of mineral and organic fertilizers give the best results in terms of grape yield (4.7 kg vine^{-1}) and the physical and chemical characteristics of bunches and berries (14.72% sugar) [70,71]. Only individual application of organic fertilizers led to the lower yield (3.6 kg vine^{-1}) [70,72] and lower content of sugar in berries (14.29%) [70,73], as well as to the increase in berry acidity (4.32 g L^{-1}) [70]. The overall polyphenol concentration is higher in organic grapes, resulting in a higher protection from oxidation [72]. The fertilization practice, based on exclusively inorganic fertilizers, could jeopardize soil quality and content of SOC.

Table 5 shows the SOC concentration in vineyards with different fertilization strategies. Combining ameliorative fertilization and application of farmyard manure initially with continuous application of NPK inorganic fertilizers has led to the highest SOC. In relation to the plots where no fertilizer was applied, the SOC content was increased by 0.37%, while in relation to the application of foliar fertilizer only, it was increased by 0.27%. These differences were statistically significant, while there were no statistically significant differences compared to the other variants. Similar results were obtained by other authors. Yang et al. [74] indicated that the SOC content could be maintained at a relatively stable level under sufficient chemical fertilizer application without return of manure and crop residue conditions, and SOC content was increased with the combination of chemical fertilizer and manure application.

Table 5. Influence of different fertilization strategies on SOC concentrations (%) of vineyards (0–30 cm and 30–60 cm).

Fertilization Strategies ¹		SOC (%)		Mean SOC (%)
		0–30 cm	30–60 cm	
1	AF + FM + NPK	1.15	1.01	1.08 a
2	AF + NPK	1.14	0.97	1.05 ab
3	AF + FM	0.94	0.81	0.88 abc
4	FM + GM	1.12	0.89	1.00 ab
5	F	0.87	0.75	0.81 bc
6	NF	0.85	0.58	0.71 c

¹ AF—ameliorative fertilization initially; FM—farmyard manure initially; GM—green manure; NPK—continuous application of nitrogen, potassium and phosphorus inorganic fertilizers; F—foliar fertilization; NF—no fertilizers. Significant differences are labeled with different letters (Duncan's range test, $p < 0.05$).

Application of AF + NPK led to the SOC concentration that was statistically significantly higher than in the variant without fertilization, by 0.34%. Similar results were obtained by application of FM + GM, where SOC was increased by 0.29%, compared to the NF. There were no statistically significant differences compared to other variants.

The lowest SOC content was recorded on plots that had not been fertilized at all, as well as those that had been fertilized with only foliar fertilizer. Similar results were observed by Liu et al. [75]. Hao et al. [76] stated that the effects of manure application, tillage, crop rotation, fertilizer rate, and soil and water conservation farming have positive influence on the SOC pool. They found that SOC at the 0–15 cm soil layer was 6.2%, 7.7% and 9.3% higher with manure, chemical fertilizers and manure plus fertilizers, respectively, than with no fertilizer application.

Between the vineyard establishment and 2015, the mean SOC concentration decreased in both depths of unfertilized vineyards (Table 6). In fertilized vineyards, SOC decreased only in topsoil. In this, the shallow layer, tillage significantly affects soil respiration, temperature, water content and other soil properties. The contact between soil and crop residues increases and organic matter is more exposed to microbial attack. This leads to a decrease in the content of SOC.

The reduction of SOC was rapid in the earlier stage of cultivation. Similar results were obtained by other authors. Liu et al. [75] showed a significant decline of total SOC that occurred in the first five years of cultivation where the average SOC loss per year was about 2.3 t ha⁻¹ for the 0–17 cm horizon. The average annual SOC loss between 5- and 14-year cultivation was 0.95 t ha⁻¹ and between 14- and 50-year cultivation it was 0.29 t ha⁻¹. Compared with the uncultivated soil, Liu et al. also indicated that SOC loss (the sum of three horizons) was 17%, 28% and 55% in the 5-, 14- and 50-year cultivation periods, respectively. Biddoccu et al. [77] found that average soil loss in a mountain vineyard, Aosta valley (NW Italy), was 15.7 t ha⁻¹ y⁻¹. The loss of the SOC could be reduced by taking into account some of the different mitigation options, such as manuring and fertilizing, conservation tillage, management of crop residues and cover cropping [54,78,79].

Table 6. Soil organic carbon change in vineyards between the establishment and 2015.

Fertilization Strategies ¹	Years of Cultivation	SOC Concentration Change (g kg ⁻¹ y ⁻¹)		SOC Stock Change (t ha ⁻¹ y ⁻¹)		Rate of SOC Stock Change (% y ⁻¹)	
		0–30 cm	30–60 cm	0–30 cm	30–60 cm	0–30 cm	30–60 cm
NF	≤5	−0.77	−1.15	−3.61	−5.21	−8.42	−10.21
IF + FM	≤5	−1.66	0.47	−6.75	2.01	−13.29	6.00
	6–40	−0.10	0.10	−0.42	0.43	−0.64	1.17

¹ NF—no fertilizers, IF—inorganic fertilizers continuously, FM—farmyard manure initially.

The reduction of SOC in topsoil of fertilized vineyards, in the first five years, was higher compared to the unfertilized plots. The reason is initially higher concentration of SOC in fertilized vineyards. Similar results were obtained Garcia-Diaz et al. [80]. They stated that the decrease in SOC content after tillage was greater in the treatment that presented higher SOC content.

The SOC content increased in subsoil of vineyards that had been fertilized initially with sufficient amounts of inorganic fertilizer during the cultivation and farmyard manure. The deep tillage (60–80 cm) has led to deep placement of organic amendments and equalization of SOC content between the mixed layers. On average, even 35 years after deep tillage event, the subsoil still contained 13.49 t ha⁻¹ more SOC than before this measure. It can be concluded that deep tillage can preserve SOC in the deeper soil layer and prevent carbon loss from the surface layer. Subsoil holds a large potential to store additional soil organic carbon (SOC) because of the large number of unsaturated mineral surfaces and environmental conditions that impede SOC decomposition, e.g., more constant moisture and temperature regime or oxygen limitation [81]. Similar results were obtained by other authors. According to Liu et al. [75], deep tillage (subsoiling) increased SOC and N relative to conventional tillage. Cervantes et al. [81] stated that after the deep plowing event, the layer of the deeply plowed fields accumulated on average 0.4 ± 0.1 Mg SOC ha⁻¹ yr⁻¹.

Similar results were stated by Liu et al. [75] with the rotary plowing and conventional tillage, where the SOC contents at 16–30 cm were higher than in the depth between 0 and 15 cm, indicating that more root residues were incorporated into this layer. This result was consistent with mixing of organic matter by plowing, but opposite to results with no-tillage practice or conservation tillage [82–84]. According to Campbell et al. [85], SOC gains under no-till were about 250 kg ha⁻¹ yr⁻¹ greater than for tilled systems, regardless of cropping frequency. Within the surface 7.5 cm, the no-till system possessed significantly more SOC (by 7.28 t ha⁻¹) relative to the conventional tillage [86].

3.6. Correlation of SOC with Soil Properties

The dependence of SOC content and other physical and chemical properties of soil was examined by correlation analysis and shown in Figure 10. A statistically significant correlation was found only in the lower undisturbed soil layers. In the upper layer, there was a significant decrease in the SOC content and disturbance of the ratio of SOC and other soil properties due to the strong anthropogenic impact. A significant positive correlation of SOC with clay content was found. The positive relationship between clay content and SOC confirmed the global relation between them [87–89]. The fine fraction of soil serves as a measure for SOC storage [66]. Smaller particle size has better water retention, fertilizer retention capacity and higher nutrient content [67]. The stability of SOC is determined by the chemical nature of SOM, absorption in the mineral part of the soil and its participation in the formation of structural microaggregates [90]. With an increased content of clay particles, the content of SOC tends to increase. The reason for this is the bond between the surface of the clay particles and OM, which slows down the decomposition process. Soils with higher clay content increase the potential for aggregate formation [51].

Macroaggregates physically protect organic molecules from further mineralization caused by microorganisms [91]. In similar climatic conditions, the SOM content in fine-textured (clay) soils is two to four times higher than in coarse-textured (sandy) soils [92]. The low clay content of the soil tends to be poor in soil aggregation stability and water holding, due to low cohesion forces between elementary particles that affect the porosity [6].

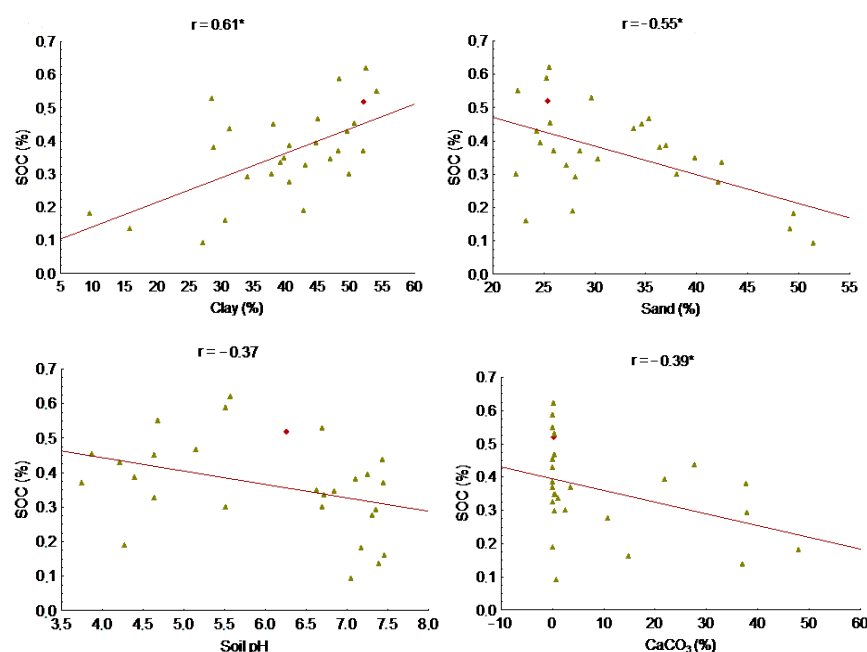


Figure 10. Correlation of SOC with soil properties: clay, sand, CaCO_3 content and pH value, in subsoil layers (>60 cm) of vineyards; * $p < 0.05$.

Sand content was significantly negatively correlated with SOC content, which agrees with the results of Li et al. [93]. They concluded that increasing desertification would reduce the accumulation of SOC. Sandy soils usually contain less SOM than soils with a finer texture, such as loam or clay. Lower moisture content and higher aeration in sandy soils results in faster SOM oxidation compared to heavier soils. In general, poorly drained soils have a higher moisture content and poorer aeration. This results in a higher organic matter (OM) content in these soils than in their better-drained equivalents [51].

SOC content decreased with increasing pH and CaCO_3 content. Negative correlation between pH value and SOC was found in the study of Islam et al. [94], especially in the presence of high sand percentage and high concentrations of Na^+ . Similar results were obtained by Ayaz et al. [95]. In their examination, the soil organic carbon stock negatively correlated with soil pH ($r = -0.38$, $p \leq 0.05$) and calcium carbonate ($r = -0.45$, $p \leq 0.01$). Increase of the calcium carbonate concentration and soil pH significantly affect soil microbial activity and reduces the SOC quantity by the enhancement in the rate of mineralization.

4. Conclusions

Intensive viticulture without adequate continuous application of fertilizers combined with organic amendments leads to decreasing of SOC in topsoil of the vineyard. The SOC stock in the topsoil and subsoil of vineyards was lower than the average value for agricultural land in Serbia. The soil type was an important predictor of carbon storage in topsoil.

More efficient management is necessary to increase SOC. The return of crop residues and application of manure or other organic fertilizers could be combined into a management system to prevent the decrease of SOC, but questions arise about the effect of these practices on grape yields and quality, as well as acceptability by farmers. Systematic

monitoring and more research of long-term SOC change is needed to evaluate the effect of different fertilization strategies on the on the SOC state in vineyards.

Deep tillage has the potential to increase SOC, but the investigation into SOC storage capacity and strategies for effective deep tillage management are necessary.

Soil conservation measures, such as cover crops, conservation buffers, drainage terraces, conservation tillage etc., should be considered for improving the vineyard management.

Supplementary Materials: The following are available online at <https://www.mdpi.com/2073-4395/11/1/9/s1>, Figure S1: Some of observed vineyards in the Tri Morava region.

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