



# A field investigation on the soil management practices in a productive vineyard considering C sequestration and water resistance of soil structure

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## Abstract

Optimal soil structure is linked to soil organic matter (SOM). Different soil management practice alternates SOM but the storing C to soil structure formation in vineyard soils is not sufficiently studied. In addition, understanding the impact of soil management practices in vineyard on changes in SOM and soil structure is crucial for sustainable viticulture. In this study conducted in a productive vineyard near Nitra city, Slovakia, we investigated the dynamics of individual size-fractions of water-stable aggregates (WSA) as results of changes in soil organic matter (SOM) under different soil management practices. Five distinct management practices – Intensive tillage system (T); Intensive tillage system with poultry manure (T + M); Strip grass (G); Fertilization in lower rates of NPK (NPK 1) to grass strips; and Fertilization in higher level of NPK (NPK 2) to grass strips were adopted to be tested. The results have shown that the highest content of soil organic carbon (SOC)  $20.3 \pm 3.68 \text{ g kg}^{-1}$ , labile carbon ( $C_L$ )  $2.71 \pm 0.78 \text{ g kg}^{-1}$ , and the most favor soil structure were identified in NPK 1, however, the most effectively improved soil structure and C sequestration (SOC:  $19.9 \pm 3.88 \text{ g kg}^{-1}$ ;  $C_L$ :  $2.53 \pm 0.83 \text{ g kg}^{-1}$ ) was under G treatment. SOC had a more significant influence on the content of water-stable aggregates than  $C_L$ . Intense changes in SOM took place in larger size-fractions of water-stable macroaggregates (WSA<sub>ma</sub>) above 2 mm, while smaller WSA<sub>ma</sub> below 2 mm and water-stable microaggregates were more stable.

**Keywords** Labile carbon · NPK fertilization · Poultry manure · Soil organic carbon · Strips grass · Water-stable aggregates

## Introduction

Land degradation in vineyard lands due to climate change, excessive cultivation as well as long-term productivity of agroecosystems are one of the key reasons for exploring and optimizing land management systems which are eco-friendly

(Lazcano et al. 2020). Vineyards are often located in sloped strata and have relatively lower quality of plant available soil nutrients (Šimanský et al. 2016; Garcia et al. 2018). In these slopes, loss of macronutrients occur due to erosion, higher hydraulic gradients during rainfall, and susceptibility to desiccation cracks during summer drought (Bordoloi and Ng 2020). From the perspective of nutrition, vine plants can intake nutrients even from less available forms in contrast to conventional crops with available nutrient concentrated soil (White 2015; Súľovský and Hriník 2016). Generally, mineral soils with a higher skeleton content (soil fragments or skeleton > 2 mm) are chosen to produce quality wines and grapes, raisins, vinegar (Basalekou et al. 2023). There are also significant risks associated with such kind of soils due to their susceptibility to erosion. During intense storms and heavy rains, soil erosion is accelerated as these soils lack inherent cohesion as well as anthropogenic activities lower soil shear strength (Lieskovský and Kenderessy 2013; Rodrigo Comino et al. 2017; Garcia et al. 2018; Dugan et al. 2023). This results in a decline of the supply of soil organic

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matter (SOM), leaching of nutrients, deterioration of physical properties, and reduction of overall soil fertility (Auzet et al. 2006; Sůľovský and Hriník 2016). Even though the vineyard plants does not have high requirements for nutrients, it does not mean that its successful growth would be limited by the soil environment (White 2015; Sůľovský and Hriník 2016; Garcia et al. 2018). White (2015) emphasized the importance of soil structure in addition to soil depth, cohesion, favorable chemistry, and organic matter for successful vine growing.

Soil structure is a key attribute of soil quality and health, and it is the basis for sustainable crop production (Lal 1991; Basset et al. 2023) particularly for vineyards (White 2015; Sůľovský and Hriník 2016; Šimanský et al. 2016). By connecting and grouping soil particles into soil aggregates through various aggregation mechanisms of chemical, physical, and biological origin, the spatial arrangement of soil mass in the soil is created (Six et al. 2002; Lieskovský et al. 2024). Briefly, soil structure represents the arrangement of soil particles (organic, inorganic) and voids in the soil profile (Lal 1991; Schlüter et al. 2020; Or et al. 2021). There is a very close relationship between SOM and soil structure (Tisdall and Oades 1982; Six et al. 2004; Bronick and Lal 2005; Šimanský et al. 2022), because SOM and its fractions have a major influence on overall soil aggregation. Conventionally, the higher content of soil organic carbon (SOC), the more stable soil aggregates are formed. As reported by Levy and Mamedov (2002), a strong relationship between the water resistance of aggregates and SOC exists. To achieve low soil erodibility and favor soil structure for nutrient retention, the amount of carbon in the soil should be relatively high. For instance, mineral soils should ideally have SOC more than 4% (Levy and Mamedov 2002). Such content is very rare in vineyard soils and soils often present poor organic carbon levels (Coll et al. 2011; Salomé et al. 2016; Šimanský et al. 2016). Soil management practices in vineyard significantly interfere to the turnover of SOC (Novara et al. 2019) which ultimately alternates soil structure (Novara et al. 2019; Šimanský et al. 2022; Lieskovský et al. 2024). Intensive tillage reduces the SOC content and degrades soil structure including vineyard soils (Šimanský et al. 2016, 2022; Novara et al. 2019). Thus, from the above discussion, soil remediation strategies to improve nutrient retention and facilitate higher vine yield is essential, particularly noting the anticipated changes in weather due to climate change.

Grass strips between rows of vines are recommended to minimize soil erosion. A grassy surface of soil reduces the negative effect of raindrops, increases water infiltration (Celette et al. 2005), and increases SOM (Šimanský et al. 2016; Novara et al. 2019; Lazcano et al. 2020). Roose (1996) stated that from an ecological and economic standpoint, a

properly chosen soil tillage management in vineyards can significantly improve the growth of vines. SOM and soil structure could be modified through soil management practices. However, soil management practices implemented in standard crop production are not always suitable for vineyard soils. Moreover, not all agronomic practices are suitable for all soil-climate conditions especially in the temperate zone of Central Europe. Information about different management systems in vineyards comes mainly from Mediterranean countries (Dugan et al. 2023), and countries with advanced viticulture (Celette et al. 2005, 2008; Coll et al. 2011; Conant et al. 2017; Garcia et al. 2018; Lazcano et al. 2020). There is considerably less field assessment of soil management practices in vineyards from Central Europe, particularly Slovakia. At the same time, even within the framework of the Slovak Republic viticulture has been considered to improve the overall agricultural economy (Sůľovský and Hriník 2016). Monitoring the effects of different soil management practices on changes of soil properties in the context of viticulture is therefore essential for the Central European region. Based on long-term soil management practices in productive vineyards, it is possible to provide relevant information for winegrowers to practice optimal soil management strategies that is sustainable in long-term.

In the past, intensive soil tillage was typical for the vineyard region in which the study was carried out, which resulted in the stabilization of the SOC pool (Šimanský et al. 2023). There are inherent assumptions/hypothesis (H 1) that with continued intensive soil tillage, significant changes in the SOM will not be recorded. Second, high doses of (Nitrogen-Phosphorus-Potassium) NPK to grassy strips will accelerate SOM mineralization and subsequent C sequestration will be lower (H 2). Third, application of organic fertilizer and growing grasses between rows of vines, as well as rational NPK fertilization in grassy strips in vineyard, will increase the SOM and improve water resistance of soil structure (H 3). Fourth, SOC will have a more pronounced effect on the content of water-stable aggregates than labile carbon (H 4). To investigate these four hypotheses in vineyard conditions, the aim of this study is to explore (1.) the dynamics of aggregation due to the increase/decrease of SOC and labile C in the soil and assess individual water-stable aggregates size-fractions that may form result of the application of different soil management practices; (2.) the dynamics of incorporation of SOM into the soil and into individual size-fractions of water-stable aggregates, and (3.) discuss which soil management practices are sustainable from the viewpoint of SOM and soil structure.

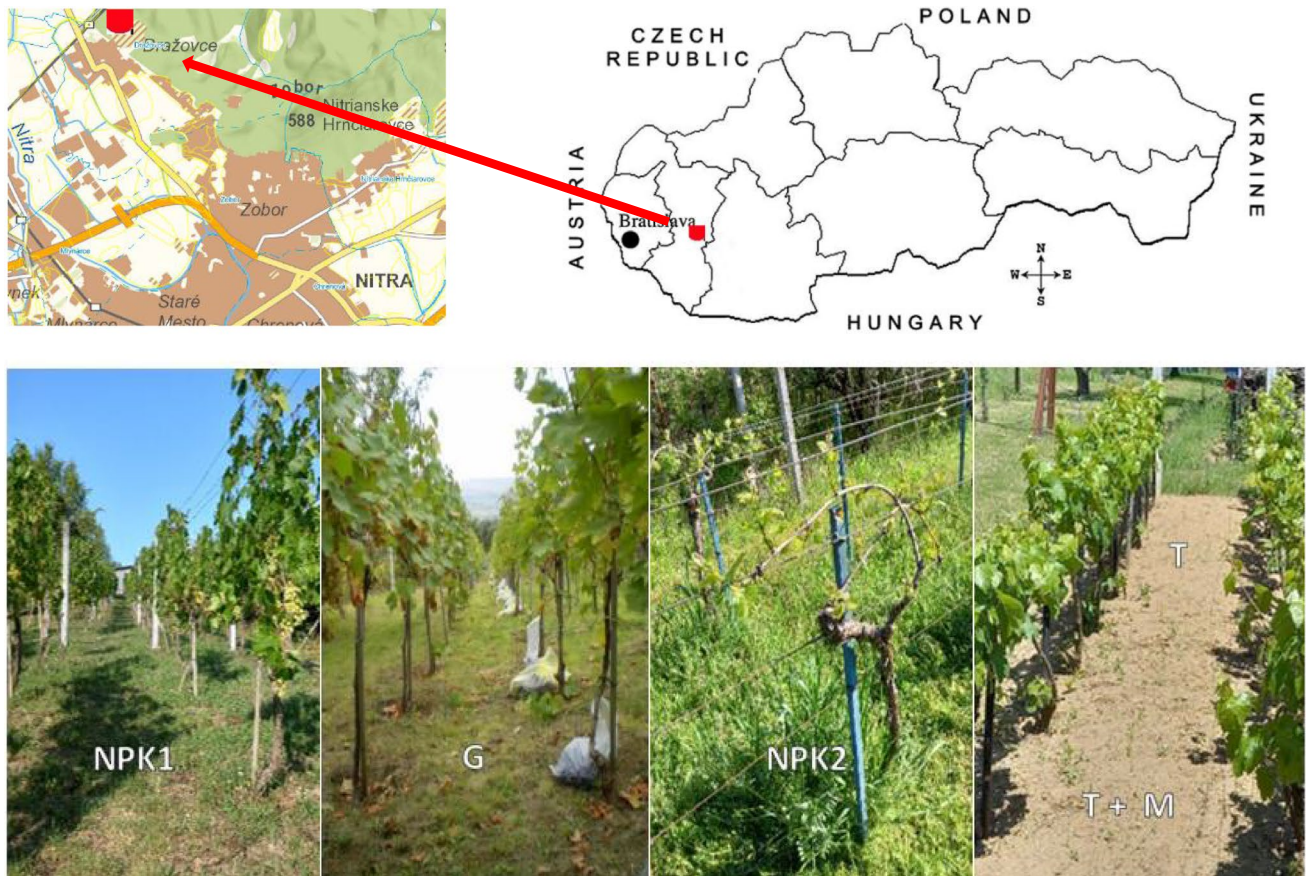
## Material and Methods

### Site description

The experimental study is located in Nitra-Dražovce (48°21'6.16"N, 18°3'37.33"E) which belongs to the Nitra viticulture region located in southwestern Slovakia in Central Europe (Fig. 1). The experimental area (vineyard) is situated in the south-western foothills of Zobor Hill. The locality has a warm temperate climate and fully humid with warm summers (Cfb) according to the classification of Köppen-Geiger (Kottek et al. 2006) with average annual temperature of 9.8 °C and average annual precipitation of 550 mm (Bochníček and Hrušková 2015). The geologic substrate of the studied area is composed of Mesozoic sedimentary rocks, predominantly Cretaceous, Jurassic, and Triassic limestones. The soil in the vineyard is classified as Rendzic Leptosol (IUSS WRB 2015) and had a sandy loam soil texture (sand: 56.9%; silt: 33.0%; clay 10.1%), a slightly alkaline reaction (pH in H<sub>2</sub>O: 7.28), and low soil organic carbon (17 g kg<sup>-1</sup>). The sorption complex was

fully saturated by basic cations (Bs: 99.3%), and the cation exchange capacity (CEC: 476 mmol kg<sup>-1</sup>) was high. Before the experiment, the soil under the vineyard had very low total N (Nt: 1067 mg kg<sup>-1</sup>) and hydrolytic acidity (Ha: 3.9 mmol kg<sup>-1</sup>), and a good supply of available P (99 mg kg<sup>-1</sup>) and K (262 mg kg<sup>-1</sup>).

The location, which also includes an experimental vineyard, was deforested in the eleventh century. At that time, vineyards were planted here, and the area was alternately used for growing vines or used for pasture (Rosinová et al. 2005). For the last 60 years, the area has been used for vine growing or fruit trees and as a horticultural area. The area in which the experimental vineyard is located was abandoned until 1999. Before the establishment of the vineyard in 2000, the area was cleared of overgrown bushes, old fruit trees, and vines and then deeply plowed to a depth of 30–35 cm. In the spring of 2001, vines (*Vitis vinifera* L. cv. Chardonnay) were planted in rows at a 2 × 1.2 m spacing. In 2001–2006, the vineyard soil was intensively loosened due to weed removal in and between the rows of vines, and every autumn, between the rows, the soil was plowed down to 25 cm.



**Fig. 1** Location of the study area and treatments (T – Intensive tillage system, T + M – Intensive tillage system with application of manure, G – Strip grass, NPK1 – Fertilization in lower rates of NPK, NPK2 – Fertilization in higher level of NPK)

## Experimental design

The experiment in productive vineyard with different soil management practices was started in 2006 and arranged in a randomized block design with three replications (consisted of five soil management practices). The characteristics of the five systems are described below:

- 1) Intensive tillage system (T): Every autumn, the soil was plowed to a depth of 25 cm and intensive loosening was done by the cultivator to a depth of 10–12 cm for regulation/removal of weeds between rows of the vine during the vegetation season of the vine, overall, three times on average. No manure and no fertilization application were done.
- 2) Intensive tillage system with application of manure (T + M): Intensive tillage system is the same as in T treatment. In 4-year cycles (2005, 2009, 2013, 2017, and 2021), in this system additional poultry manure at a dose of 40 t ha<sup>-1</sup> was plowed into the soil in autumn. The poultry manure in dry matter contained 55% organic substances, 2.8% of total nitrogen, 1.3% P (P<sub>2</sub>O<sub>5</sub>), 1.2% K (K<sub>2</sub>O), and its pH was in the range of 6 to 8.
- 3) Strip grass (G): In 2006 a mix of grasses (*Lolium perenne* L. 50% + *Poa pratensis* L. 20% + *Festuca rubra* subsp. *commutata* Gaudin 25% + *Trifolium repens* L. 5% – standard commercial mix of grass for vineyard soils at conditions of Slovakia) was sown in between rows of vines. Strip grass in vineyard was cut down during vegetation season of vines, on an average three times. The aboveground biomass of the grass remained in-situ on the surface as a typical mulch layer. No manure and no fertilization application were done.
- 4) Fertilization in lower rates of NPK (NPK 1): The vegetation applied between vine row were same sown as in G treatment. Additionally, in this management system, doses of NPK fertilizers corresponding to 100 kg ha<sup>-1</sup> N, 30 kg ha<sup>-1</sup> P, and 120 kg ha<sup>-1</sup> K were applied to the vines. The dose of nutrients was equally divided into two proportions. First half applied into the soil in the spring (bud burst) and another half at flowering (Fecenko and Ložek 2000).
- 5) Fertilization in higher level of NPK (NPK 2): The vegetation applied between vine row were same sown as in G treatment. An annual application of NPK fertilizer in higher level (125 kg ha<sup>-1</sup> N, 50 kg ha<sup>-1</sup> P, and 185 kg ha<sup>-1</sup> K). The dosage of nutrients was divided in two proportions, wherein 2/3 of the nutrients were applied into the soil in spring (bud burst: in March) and 1/3 at flowering (in May) (Fecenko and Ložek 2000).

## Soil sampling and analysis

All five soil management practices in a productive vineyard mentioned above were used to evaluate the SOM and individual size-fraction of water-stable aggregates. Soil samples were taken from each type (n = 3) of soil management from a soil depth of 0–25 cm every spring from 2009–2022. Soil samples for determining the size-fraction of water-stable aggregates (WSA) were taken with a spade to preserve the natural lines of soil aggregates (undisturbed soil samples). Soil samples (disturbed soil samples) for the determination of SOM were taken from the same areas. A set of sub-samples from each replicate was mixed into the average soil sample.

All samples were dried at room temperature and the ground samples (sieved to 0.25 mm) were determined for SOM parameters. Soil organic carbon content (SOC) in soil bulk was then established by the wet combustion method (Dziadowiec and Gonet 1999), and the labile carbon content (C<sub>L</sub>) in soil bulk was measured by the Loginow method (Loginow et al. 1987). Baksheev method was used for determination of size-fractions of WSA in undisturbed soil samples (Hrivňaková et al. 2011). The size-fractions of WSA were following: > 5, 3–0.5, 0.5–0.25 as water-stable macro-aggregates (WSA<sub>ma</sub>) and < 0.25 mm as water-stable micro-aggregates (WSA<sub>mi</sub>). In individual size-fractions of WSA, SOC and C<sub>L</sub> contents were determined the same methods than in soil bulk.

## Statistical analysis

Statistical analysis of the results included calculation of mean values of the variables and standard deviations. Statistical significance of differences between the means was tested using the Kruskal-Wallis test at significance level equal 0.05. Principal component analysis (PCA) was used to identify sources of variability and find interrelationships between variables. A simple linear regression was applied to establish the relationships between the soil characteristics and observation time (year). The statistical analysis was performed using Statistica v.13.1 (Dell Inc., Round Rock, TX, USA).

## Results

### Soil organic matter in bulk soil

SOC contents were relatively stable over time under T and T + M treatments and significantly increased under the remaining treatments (G, NPK 1, and NPK 2). No significant linear trends for C<sub>L</sub> in bulk soil were determined

for the period of 2009–2022, except for NPK 1 treatment (Fig. 2). There,  $C_L$  increased at an average speed of  $95 \text{ mg kg}^{-1} \text{ year}^{-1}$ . Correlation analysis showed that temporal changes of SOC and  $C_L$  were positively, usually strongly related in all treatments (G:  $r=0.85$ ; T:  $r=0.51$ ; T+M:  $r=0.77$ ; NPK 1:  $r=0.75$ ; NPK 2:  $r=0.79$ ). Hence  $C_L$  content was rather a function of SOC than treatment. Table 1 show statistical comparison of different soil management practices in terms of SOC and  $C_L$  contents in bulk soil considering data from all years. The analysis highlighted differences between T and remaining treatments that form homogenous group. The SOC increased in the following order  $T < T+M < \text{NPK 2} < G < \text{NPK 1}$ . With small differences, almost identical trend in increasing the  $C_L$  content in bulk soil was found. The lowest  $C_L$  content was found in T and the highest in NPK 2.

**Contents of individual size-fractions of water-stable aggregates**

During the period 2009–2022, individual size-fractions of WSA reacted very sensitively to changes in their contents, especially in treatments G, T+M and NPK 1 (Fig. 3). For this reason, it was not ideal to reliably identify a simple linear trend of increase/decrease in their contents. However, over the period of 14 years, due to intensive soil tillage (T), the contents of the WSAm<sub>5–3</sub> and 2–1 mm significantly decreased linearly, but on the other hand, the contents of the smaller WSAm<sub>1–0.25</sub> mm linearly increased. A higher rate of NPK (NPK 2) showed a linearly increase in WSAm<sub>5–2</sub> mm content and a decrease in WSAm<sub>0.5–0.25</sub> and WSAm<sub>i</sub> during a period of 14 years. Overall, considering all years of the studied period, we compared mean values of WSA contents in size-fractions to evaluate general effect of different treatments on soil aggregation (Table 2). The analysis showed that treatments T and T+M did not differ in almost all cases, showing generally lower contents of large WSAm<sub>a</sub> (above 2 mm) at higher contents of small ones (below 1 mm). The second group formed treatments G, NPK 1 and NPK 2. The group is characterized by higher contents of large WSAm<sub>a</sub> (above 2 mm) and lower contents

**Table 1** Mean ± SD contents of SOC and  $C_L$  ( $\text{mg kg}^{-1}$ ) in bulk soil for the whole period of experiment (the same letters in the columns indicate homogeneous groups according to Kruskal-Wallis test, 0.05 significance level)

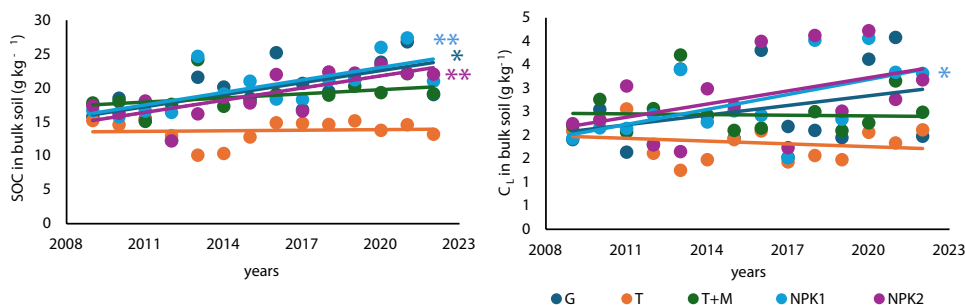
Treatment	SOC $\text{g kg}^{-1}$	$C_L$
T	$13.7 \pm 1.71^a$	$1.85 \pm 0.38^a$
T+M	$18.8 \pm 2.26^b$	$2.43 \pm 0.53^b$
G	$19.9 \pm 3.88^b$	$2.53 \pm 0.83^b$
NPK 1	$20.3 \pm 3.68^b$	$2.71 \pm 0.78^b$
NPK 2	$19.1 \pm 3.30^b$	$2.81 \pm 0.86^b$

of small WSAm<sub>a</sub> (1–0.25 mm) and WSAm<sub>i</sub> as compared to T and T+M.

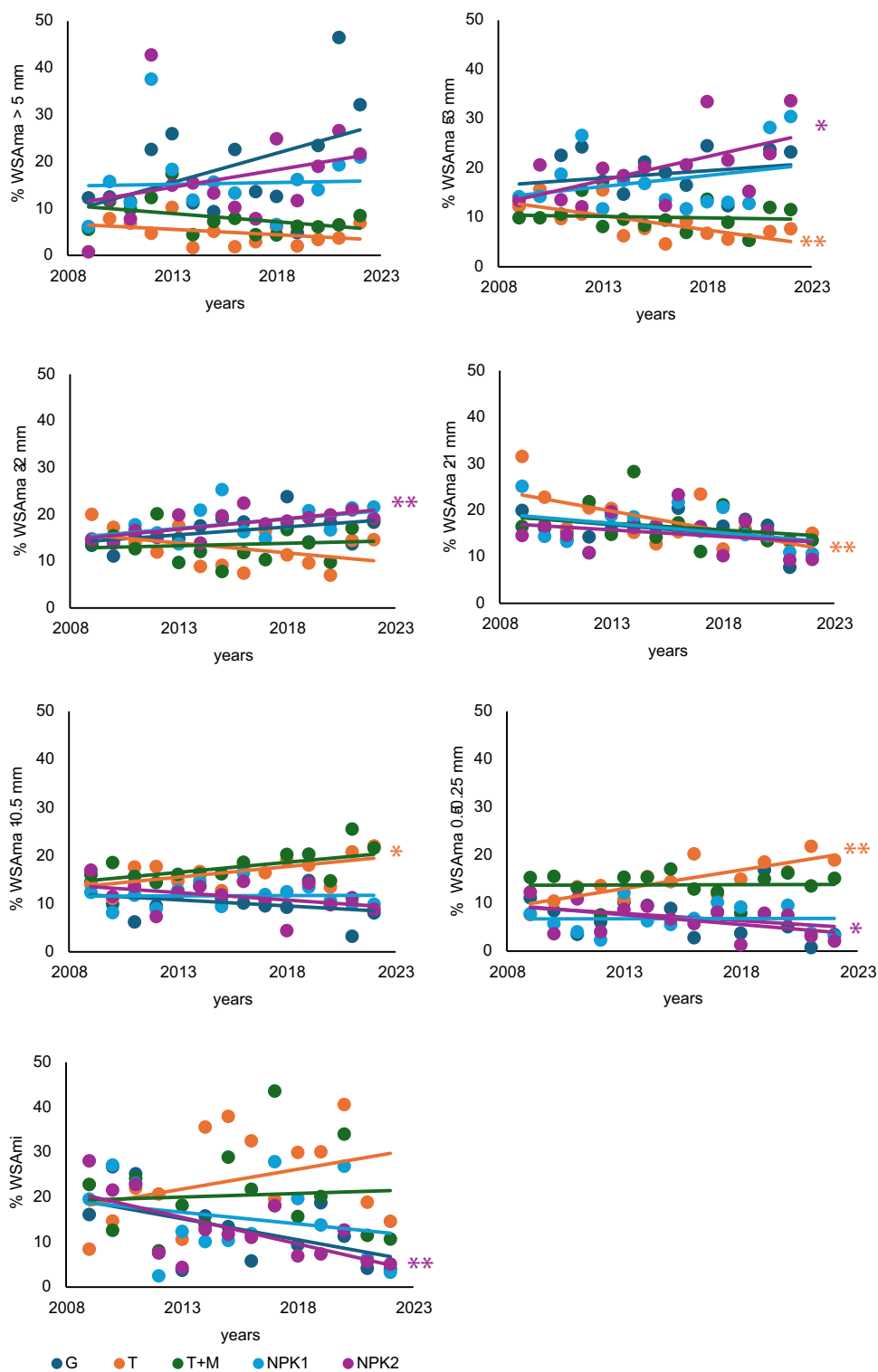
**Soil organic matter in size-fractions of water-stable aggregates**

In the T+M treatment, the SOC content in WSAm<sub>5–3</sub> mm and WSAm<sub>i</sub> increased linearly. The incorporation of SOC in WSAm<sub>5–3</sub> mm, WSAm<sub>3–2</sub> mm, and WSAm<sub>i</sub> represented an annual increase of 578, 492, and 280  $\text{mg kg}^{-1} \text{ year}^{-1}$ , respectively for a period of 14 years. At the same time, a significant linear increase in SOC in WSAm<sub>5–3</sub> mm (at an average speed of  $236 \text{ mg kg}^{-1} \text{ year}^{-1}$ ) was observed in G treatment (Fig. 4). The dynamics of  $C_L$  in WSA were relatively in wide range in all treatments during the period of 2009–2022, and therefore it was not possible to determine simple linear trends of increasing or decreasing its content (Fig. 5). Only in the T treatment, there was found a statistically significant linear decrease in  $C_L$  in WSAm<sub>2–0.5</sub> mm. Overall, treatments differed in SOM in WSA (Table 3). In WSAm<sub>a</sub>, the highest SOC contents were observed in T+M compared to the other treatments. In T treatment, SOC content in WSAm<sub>i</sub> was lower by 13, 15, 7 and 20% than in G, T+M, NPK 1 and NPK 2, respectively. Overall, higher  $C_L$  in WSAm<sub>a</sub> in the case of T+M compared to other treatments was observed.  $C_L$  in WSAm<sub>i</sub> did not significantly differ depending on the soil management practices in a productive vineyard.

**Fig. 2** Dynamics of soil organic carbon and labile carbon in the studied treatments during 2009–2022 (\*  $p < 0.05$ ; \*\*  $p < 0.01$ )



**Fig. 3** Dynamics of individual size-fractions of water-stable aggregates in the studied treatments during 2009–2022 (\*  $p < 0.05$ ; \*\*  $p < 0.01$ )



**Relationships between soil organic matter and size-fraction of water-stable aggregates**

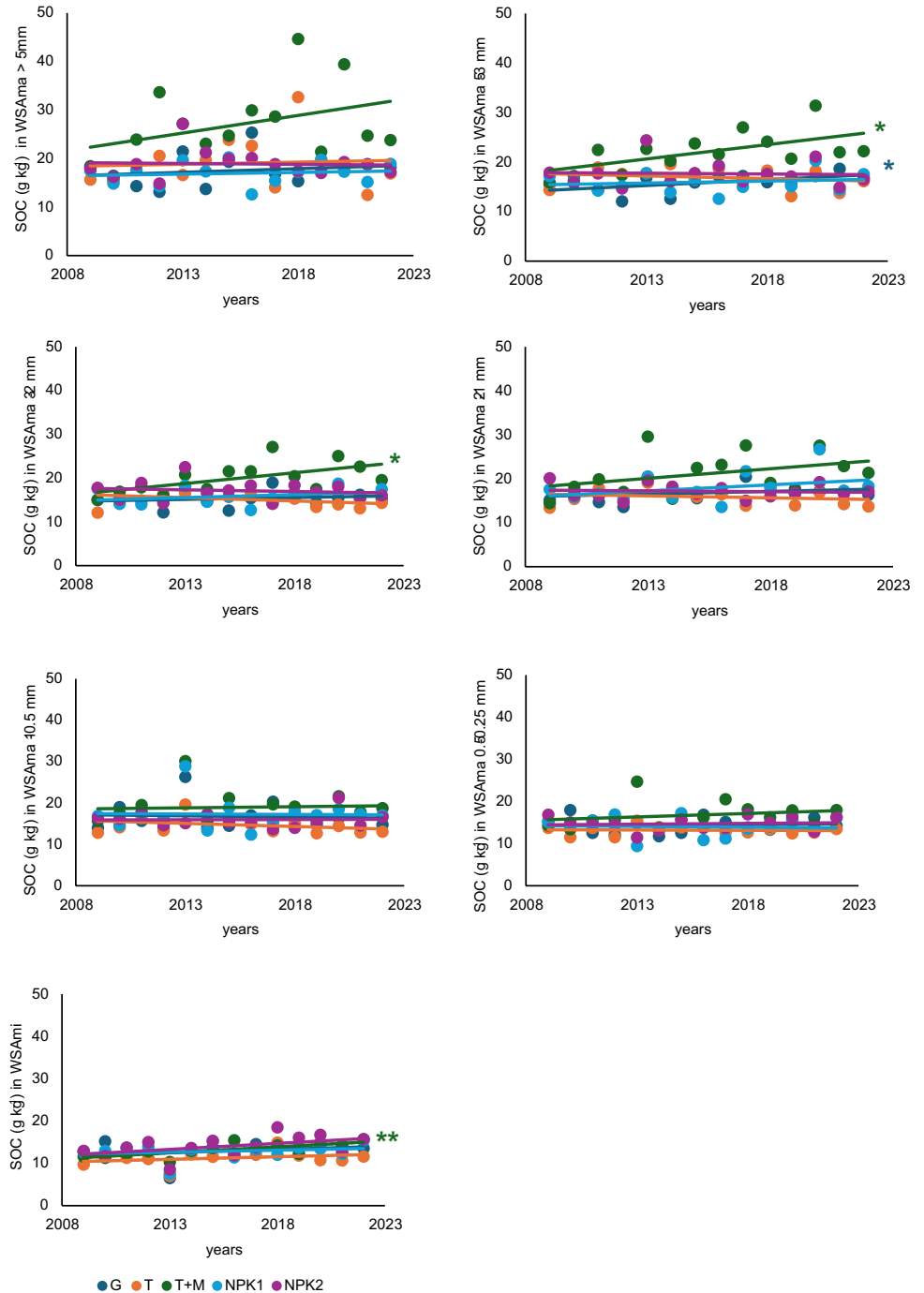
Principle component analysis based on WSA contents and contents of SOC and  $C_L$  in WSA size-fractions showed close interrelationships between the studied variables

(Fig. 6). Two major principal components explain 94% of the observed variability. Based on the results, a clear difference between large WSAm ( $> 5$  mm, 5–3 mm and 3–2 mm) and small WSAm can be observed. PC1 was strongly positively correlated with large WSAm content (above 2 mm), whereas PC2 with the content of small WSAm (below

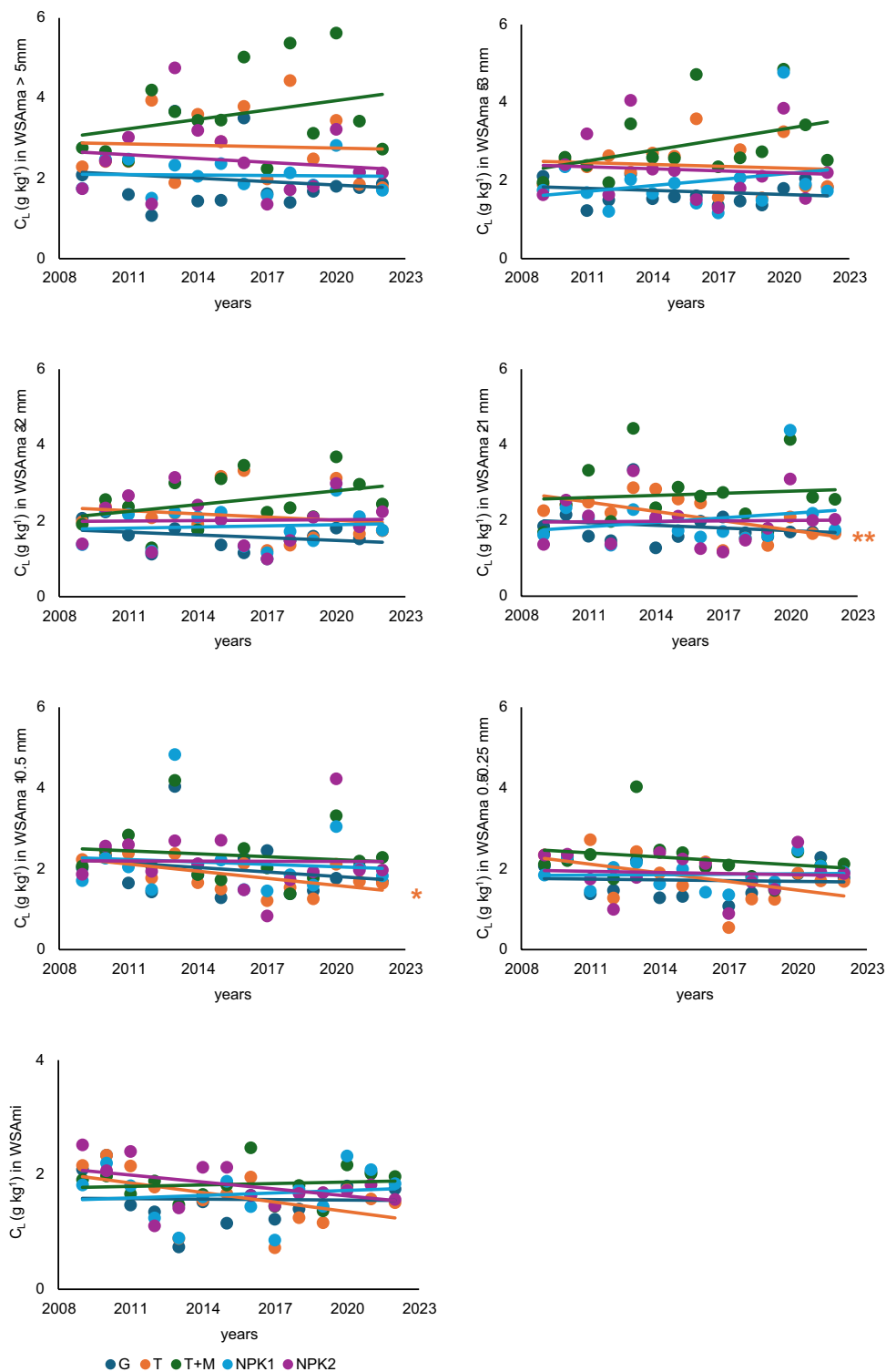
**Table 2** Mean  $\pm$  SD values of water-stable aggregates (WSA) contents of size-fractions in % (the same letters in the columns indicate homogeneous groups according to Kruskal-Wallis test, 0.05 significance level)

Treatment	Size-fraction of water-stable aggregates (mm)						
	>5	5–3	3–2	2–1	1–0.5	0.5–0.25	<0.25
	WSA <sub>ma</sub>						WSA <sub>mi</sub>
T	5.0 $\pm$ 2.5 <sup>a</sup>	8.9 $\pm$ 3.6 <sup>a</sup>	12.8 $\pm$ 4.1 <sup>a</sup>	17.7 $\pm$ 5.5 <sup>a</sup>	16.6 $\pm$ 3.1 <sup>bc</sup>	15.0 $\pm$ 4.1 <sup>b</sup>	24.0 $\pm$ 10.4 <sup>b</sup>
T+M	8.1 $\pm$ 3.7 <sup>a</sup>	10.1 $\pm$ 2.6 <sup>a</sup>	13.6 $\pm$ 3.7 <sup>a</sup>	16.5 $\pm$ 4.5 <sup>a</sup>	17.6 $\pm$ 3.6 <sup>c</sup>	13.8 $\pm$ 2.9 <sup>b</sup>	20.4 $\pm$ 9.9 <sup>bc</sup>
G	18.7 $\pm$ 11.1 <sup>b</sup>	18.7 $\pm$ 4.5 <sup>b</sup>	16.5 $\pm$ 3.2 <sup>ab</sup>	15.9 $\pm$ 3.3 <sup>a</sup>	10.2 $\pm$ 3.1 <sup>a</sup>	7.1 $\pm$ 4.3 <sup>a</sup>	12.9 $\pm$ 7.6 <sup>a</sup>
NPK 1	15.4 $\pm$ 7.9 <sup>b</sup>	17.4 $\pm$ 6.4 <sup>b</sup>	18.0 $\pm$ 3.4 <sup>b</sup>	16.1 $\pm$ 4.4 <sup>a</sup>	11.7 $\pm$ 2.3 <sup>a</sup>	6.8 $\pm$ 2.9 <sup>a</sup>	15.3 $\pm$ 8.7 <sup>ab</sup>
NPK 2	16.4 $\pm$ 10.3 <sup>b</sup>	19.9 $\pm$ 6.8 <sup>b</sup>	18.0 $\pm$ 2.7 <sup>b</sup>	15.1 $\pm$ 4.0 <sup>a</sup>	11.6 $\pm$ 3.2 <sup>a</sup>	6.6 $\pm$ 3.3 <sup>a</sup>	12.6 $\pm$ 7.4 <sup>a</sup>

**Fig. 4** Dynamics of soil organic carbon in size-fractions of WSA in the studied treatments during 2009–2022 (\*  $p < 0.05$ ; \*\*  $p < 0.01$ )



**Fig. 5** Dynamics of labile carbon in size-fractions of WSA in the studied treatments during 2009–2022 (\*  $p < 0.05$ ; \*\*  $p < 0.01$ )



2 mm) and WSAmi. Moreover, PC1 correlates strongly negatively with SOC (> 3 mm) and  $C_L$  (> 1 mm) in WSAm. It means, that large WSAm (above 2 mm) and content of SOC and  $C_L$  are more dynamic over time when compared to WSAm < 2 mm, which are more stable. Correlation matrix of PCA analysis showed that both SOC and  $C_L$  in

large WSAm are strongly negatively correlated with the content of aggregates.

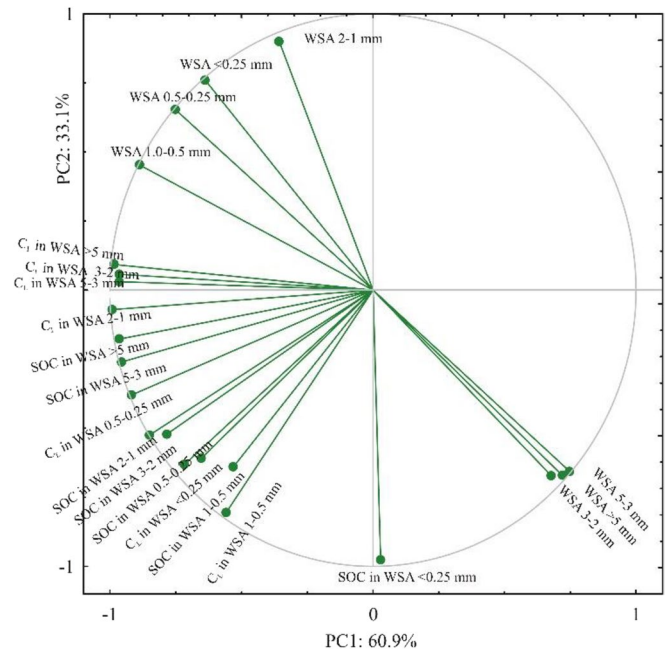
To identify rapid reaction in soil aggregation on SOC and  $C_L$  changes in bulk soil, we correlated relative (year-to-year) changes in WSA contents in size fractions with SOC and  $C_L$  (Table 4). Analysis showed both positive



**Table 3** Mean ± SD values of SOC and C<sub>L</sub> in WSA size-fractions (the same letters in the columns indicate homogeneous groups according to Kruskal-Wallis test, 0.05 significance level) – full study period

Treatment	Size-fraction of water-stable aggregates (mm)						
	>5	5–3	3–2	2–1	1–0.5	0.5–0.25	<0.25
	WSA <sub>ma</sub>						WSA <sub>mi</sub>
<b>SOC (g kg<sup>-1</sup>)</b>							
T	19.0 ± 5.03 <sup>a</sup>	16.9 ± 1.99 <sup>a</sup>	15.1 ± 1.68 <sup>a</sup>	15.9 ± 1.95 <sup>a</sup>	14.7 ± 2.02 <sup>a</sup>	13.2 ± 1.05 <sup>a</sup>	11.3 ± 1.67 <sup>a</sup>
T+M	27.1 ± 7.82 <sup>b</sup>	22.1 ± 4.00 <sup>b</sup>	19.9 ± 3.46 <sup>b</sup>	21.2 ± 4.66 <sup>b</sup>	18.9 ± 3.78 <sup>b</sup>	16.7 ± 0.30 <sup>b</sup>	13.2 ± 1.58 <sup>bc</sup>
G	17.5 ± 3.19 <sup>a</sup>	15.9 ± 1.81 <sup>a</sup>	15.3 ± 1.79 <sup>a</sup>	16.8 ± 1.89 <sup>a</sup>	16.7 ± 3.36 <sup>ac</sup>	14.4 ± 1.88 <sup>ac</sup>	12.9 ± 2.12 <sup>cd</sup>
NPK 1	17.0 ± 2.26 <sup>a</sup>	15.9 ± 1.10 <sup>a</sup>	15.9 ± 1.77 <sup>ac</sup>	18.0 ± 3.25 <sup>ab</sup>	17.3 ± 3.83 <sup>bc</sup>	13.4 ± 2.30 <sup>ac</sup>	12.0 ± 1.89 <sup>bc</sup>
NPK 2	18.9 ± 2.89 <sup>a</sup>	17.7 ± 2.57 <sup>a</sup>	17.1 ± 2.17 <sup>bc</sup>	17.2 ± 1.66 <sup>a</sup>	16.0 ± 1.93 <sup>ac</sup>	14.7 ± 1.64 <sup>bc</sup>	14.0 ± 2.49 <sup>bd</sup>
<b>C<sub>L</sub> (g kg<sup>-1</sup>)</b>							
T	2.81 ± 0.88 <sup>bc</sup>	2.39 ± 0.61 <sup>bc</sup>	2.14 ± 0.67 <sup>bc</sup>	2.12 ± 0.54 <sup>ab</sup>	1.85 ± 0.42 <sup>a</sup>	1.79 ± 0.59 <sup>a</sup>	1.61 ± 0.48 <sup>a</sup>
T+M	3.59 ± 1.09 <sup>b</sup>	2.91 ± 0.91 <sup>c</sup>	2.52 ± 0.67 <sup>b</sup>	2.70 ± 0.81 <sup>b</sup>	2.34 ± 0.72 <sup>a</sup>	2.24 ± 0.59 <sup>b</sup>	1.84 ± 0.30 <sup>a</sup>
G	1.96 ± 0.77 <sup>a</sup>	1.72 ± 0.36 <sup>a</sup>	1.59 ± 0.35 <sup>a</sup>	1.84 ± 0.49 <sup>a</sup>	1.98 ± 0.70 <sup>a</sup>	1.71 ± 0.41 <sup>a</sup>	1.57 ± 0.42 <sup>a</sup>
NPK 1	2.08 ± 0.38 <sup>a</sup>	1.95 ± 0.88 <sup>a</sup>	1.85 ± 0.67 <sup>ac</sup>	2.01 ± 0.75 <sup>a</sup>	2.14 ± 0.88 <sup>a</sup>	1.86 ± 0.34 <sup>ab</sup>	1.66 ± 0.44 <sup>a</sup>
NPK 2	2.45 ± 0.92 <sup>ac</sup>	2.28 ± 0.86 <sup>ab</sup>	2.01 ± 0.67 <sup>ab</sup>	1.98 ± 0.66 <sup>a</sup>	2.19 ± 0.78 <sup>a</sup>	1.89 ± 0.52 <sup>ab</sup>	1.81 ± 0.34 <sup>a</sup>

**Fig. 6** Principle component analysis based on WSA contents and contents of SOC and CL in WSA size-fractions



and negative correlations. There were stronger correlations in large WSA<sub>ma</sub> > 5 mm as compared to small ones (WSA<sub>ma</sub> < 2 mm) in case of SOC. In case of C<sub>L</sub> was observed large variability depending on the treatment. Correlations were poorest when all treatments were included in the same analyzed set. It means, that treatment is an important factor in short-term responses of soil aggregation to changes in SOC and C<sub>L</sub> in bulk soil.

## Discussion

### Soil organic matter in a bulk soil

Soil management practices, including these ones in vineyards, can significantly affect SOM (Celette et al. 2005; Coll et al. 2011; López-Vicente et al. 2020; Šimanský et al.

**Table 4** Correlation coefficients between relative changes (year-to-year) in contents of WSA size-fractions and SOC and C<sub>L</sub> in bulk soil (green color indicates positive correlation; red color indicates negative correlation; color intensity reflects strength of correlation)

Treatments		Size fraction of water-stable aggregates (mm)						
		>5	5-3	3-2	2-1	1-0.5	0.5-0.25	<0.25
		WSA ma						WSA mi
T	SOC	-0.306	-0.367	-0.101	-0.091	0.366	0.436	0.037
T+M		0.752	-0.067	-0.116	-0.289	0.485	0.180	-0.242
G		0.540	-0.270	-0.401	0.047	-0.237	-0.166	-0.145
NPK1		-0.294	-0.433	-0.155	0.327	-0.066	0.598	0.220
NPK2		-0.384	-0.039	0.367	0.358	0.301	0.178	0.088
All treatments		0.047	-0.213	-0.113	0.059	0.130	0.144	-0.035
		Size fraction of water-stable aggregates (mm)						
		>5	5-3	3-2	2-1	1-0.5	0.5-0.25	<0.25
		WSA ma						WSA mi
T	C <sub>L</sub>	0.042	-0.409	-0.501	-0.583	-0.053	0.289	0.531
T+M		0.648	0.198	0.127	0.014	0.623	-0.077	-0.493
G		0.759	-0.067	-0.214	0.035	-0.328	-0.408	-0.442
NPK1		-0.160	-0.277	-0.259	0.518	-0.050	0.353	0.140
NPK2		0.091	-0.065	0.109	0.020	-0.212	-0.148	0.063
All treatments		0.248	-0.095	-0.105	0.065	-0.044	-0.095	-0.095

2016; Carpio et al. 2023). The results of this study showed that overall the lowest values of SOC and C<sub>L</sub> in bulk soil were in T treatment and strip grass between rows of vines as well as grassing together with mineral fertilization increases SOM (Table 1). Intensive tillage supports mineralization processes in the soil and result in overall decline of SOM (Novara et al. 2019). As a result of long-term tillage, a balance has been established between inputs, transformation, stabilization, and output in SOM. In other words, if the supply of organic matter to the soil is maintained for a long time, through crop residues, then the SOC content does not fundamentally change. Changes in SOM fractions carried out their course over time which results in soil carbon content reaching a new equilibrium (Lal 2004). In the T treatment, for this reason, the dynamics of SOC (representing the more stable component of SOM) did not change significantly and was in balance during the 14 years of monitoring (Fig. 2). This is linked to a great amount of C that annually leaves the soil to other sinks (Richter et al. 2007). As reported by Erda et al. (1997), about 90% of C entering tilled soil comes out of that soil within a year. On the other hand, in treatments of strip grass between rows of vines (G), and application of both NPK levels (NPK 1 and NPK 2) to the strip grass; there is a built up a SOC at an average speed of 95, 99 and 99 mg kg<sup>-1</sup> year<sup>-1</sup>, respectively. This means during the years 2009–2022, a percentage increase of 20, 26 and 26 SOC was observed, respectively (Fig. 2). At the same time, in

NPK 1 treatment, C<sub>L</sub> in soil bulk increased at an average speed of 95 mg kg<sup>-1</sup> year<sup>-1</sup> for period of 2009–2022. Changes in C<sub>L</sub> were more sensitive than SOC due to different soil management in the vineyard, which is not surprising because of relatively wide range of C<sub>L</sub> dynamics in and between individual treatments. Intense changes in SOC and C<sub>L</sub> in soil bulk were in the strip grass and NPK fertilization between rows of vines treatments than in the case of tillage treatment. This confirms the first hypothesis H 1 (i.e., in intensive soil tillage treatment do not cause significant changes in the SOM), of course unless the current balance is disturbed. The above-ground and root biomass of grasses grown in between rows of vines represents a significant contribution to the SOM content (Lazcano et al. 2020). In addition, there is the production of root exudates and no-tillage, so higher production of biomass is the prerequisite for higher SOM accumulation in soil (Yang et al. 2021). Grassing degraded soils with a low SOM content can thus be used as a scenario suitable for the restoration of these soils (Conant et al. 2017), including vineyards soils (Brunori et al. 2016; Šimanský et al. 2023). Higher biomass production can be obtained through higher inputs of supplied nutrients to the soil. Long-term mineral fertilization is the source of nutrients and with subsequent increases in the formation of plant biomass, including the microbial biota, it implies that it can increase SOC (Luo et al. 2015). However, high doses of NPK may not necessarily translate into the highest observable

increase in SOC. High NPK doses support the formation of above-ground and root biomass of grasses, as well as more exudates. On the other hand, current study showed more intense mineralization of SOM (highest  $C_L$  values). Overall, the result is lower sequestration of C compared to G and NPK 1 treatments (Table 1). On the contrary, lower doses of NPK (NPK 1) in grassed vine strips support SOM humification (Šimanský et al. 2022) and thus SOC and  $C_L$  stabilization with its subsequent highest increase (Table 1; Fig. 2). The stated findings confirm hypothesis 2 (H 2).

### Contents of individual size-fractions of water-stable aggregates

In soil, optimal physical conditions are developed when in soil volume the content of size-fraction of aggregates (0.5–3 mm) dominant, and especially if mentioned aggregate size-fractions are water resistance. Based on the content of individual WSA size-fractions, it is possible to directly evaluate the soil structure (Šimanský 2014). These data can be used also indirectly to calculate other soil structure parameters such as index stability of aggregates, vulnerability of soil structure etc. (Shukla and Lal 2004). Agro-technical operations and environmental changes modify the soil structure (Bronick and Lal 2005) including vineyard soils (White 2015; Sůľovský and Hriník 2016; Šimanský et al. 2016). Intensive soil tillage systems disrupt soil aggregates, compacts soil, and disturbs plant and animal communities that contribute to the aggregation and decrease of SOM (Šimanský et al. 2016). The results of this study showed that over the period of 14 years, because of intensive tillage (T treatment), the contents of the WS<sub>Ama</sub> 5–3 and 2–1 mm size-fractions significantly decreased, but on the other hand, the contents of the smaller WS<sub>Ama</sub> 1–0.25 mm increased (Fig. 3). In addition to the intensive tillage system, the incorporation of poultry manure (T + M treatment) increased WS<sub>Ami</sub> and decreased WS<sub>Ama</sub>. Soils without the application of manure usually contain less SOC and microbial biomass, they have higher density and aggregates are resistant to the destruction only in dry conditions and less in wet conditions (Munkholm et al. 2002). Poultry manure (T + M treatment) contains a high content of labile organic matter (Kacprzak et al. 2022) – content of  $C_L$  in soil above 2 g kg<sup>-1</sup> (Table 1), which is an important binding agent, but unfortunately only temporary effect. So, in terms of long-term aggregate stability, it often has no significant effect. Comparison of individual WSA size-fractions between T and T + M treatments did not indicate any significant change. But the trend in the overall content of individual WSA size-fractions was shown in favor of T + M compared to T (Table 2). Growing grass on the inter-rows of the vine improves aggregation (Celette et al. 2005; Lazcano et al. 2020) which is also documented by the results of this study

(Table 2), especially in reducing WS<sub>Ama</sub> 2–1 mm as well as in the content of smaller WS<sub>Ama</sub> 1–0.25 mm and WS<sub>Ami</sub>. This effect can be based on the length and density of the root system as reported Rillig et al. (2002). In the case of this study, positive effect of grass strips between vine may be linked with production of root exudates (Czarnes et al. 2000), but also the interaction with the soil fauna (Bronick and Lal 2005). Rational mineral fertilization improves the soil structure in vineyard soils (Šimanský et al. 2022). In case of this study, the addition of NPK fertilization to grass strips at a lower rate (NPK 1) had a positive effect on increase of the WS<sub>Ama</sub> > 2 mm compared to T and T + M treatments, but no effect compared to G, which confirms the hypothesis (H 3) only partially. An explanation can consist of the highest content of  $C_L$  in both treatments with NPK fertilization (Table 1). The supplied nutrients had a direct effect on the development of above grass biomass (see Fig. 1: in NPK 2 it is possible to see an increased production of above-ground biomass after NPK fertilization) and roots grown in between rows of vines with a larger production of root exudates, which also triggered more intensive biological activity (Luo et al. 2015). Microorganisms produce polysaccharides that act as temporary binding agents of soil particles, microaggregates, and smaller macroaggregates into larger macroaggregates (Bronick and Lal 2005), which in the long term does not have a significant effect on the soil structure.

### Soil organic matter in water-stable aggregates

In general, SOC decreases due to long-term intensive tillage system (Khorramdel et al. 2013), however, in the case of this study, SOC remained balanced in the T treatment over a period of 14 years (Fig. 2), but overall, the lowest compared to the other treatments (Table 1). As discussed above, this result is a consequence of the stabilization of SOM dynamics due to tillage (Lal 2004) that took place several centuries ago in this site (deforestation and establishment of vineyards in the eleventh century and their intensive cultivation for the last 60 years – Rosinová et al. 2005). Intensive cultivation of the soil in the T treatment had no significant effect on changes dynamic in SOC in WSA (Fig. 4). However, the  $C_L$  in WS<sub>Ama</sub> significantly decreased at an average rate of 82 and 59 mg kg<sup>-1</sup> year<sup>-1</sup> across the size-fractions 2–1 mm and 1–0.5 mm, respectively. Expressed as a percentage, there is a decrease of 25 and 26% of  $C_L$  in these size-fractions of WS<sub>Ama</sub> over the period of 14 years. The decrease of  $C_L$  in WS<sub>Ama</sub> 2–0.5 mm influenced the increase of WS<sub>Ami</sub> content (Table 2) in the T treatment. Also, an increase of  $C_L$  in WS<sub>Ama</sub> of > 2 mm was found, indicating the formation of WS<sub>Ami</sub> inside the larger WS<sub>Ama</sub> by a fine fraction of particulate organic matter – mechanism described by Six et al. (2000). In addition, usually,  $C_L$  represents around 10% of SOC (Table 1 and 3). This means that the overall neutral

change in SOC in soil bulk is compensated by the greater  $C_L$  content in WSAm<sub>a</sub> > 2 mm in the T treatment. Contrary, application of poultry manure (40 t ha<sup>-1</sup>) significantly built-up SOC in WSAm<sub>a</sub> at an average rate of 578 and 492 mg kg<sup>-1</sup> year<sup>-1</sup> for only the size fractions 5–3 mm and 3–2 mm. At the same time, significant built-up SOC in WSAm<sub>i</sub> at an average rate of 280 mg kg<sup>-1</sup> year<sup>-1</sup> was found in T + M treatment for 14 years (Fig. 4). Applied poultry manure to the soil caused soil microorganisms to be incorporated into WSAm<sub>a</sub> and form coarse intra-aggregate particulate organic matter in them. Subsequently, during the formation of soil structure by microbial activity and other exogenous factors, coarse intra-aggregate particulate organic matter (IaPOM) was broken down into finer fractions of IaPOM within WSAm<sub>a</sub>. The fine fraction of IaPOM was bonded with the mineral particles surrounding it which resulted in a new WSAm<sub>i</sub> creation. A newly created WSAm<sub>i</sub> can be connected to another WSAm<sub>i</sub> within WSAm<sub>a</sub>, or the original stable WSAm<sub>i</sub> or WSAm<sub>a</sub> to form WSAm<sub>a</sub> (Tisdall and Oades 1982; Bronick and Lal 2005). Grassing and mineral fertilization increased SOC in bulk soil (Table 1), but in the case of SOM in WSA differences were found (Table 3). Mineral fertilization to strip grass between rows of vines did not have a significant effect on the dynamics of SOM in WSA. The results of this study (Fig. 4) showed that strip grass significantly builds up a SOC in WSAm<sub>a</sub> 5–3 mm at an average speed of 236 mg kg<sup>-1</sup> year<sup>-1</sup> which means during the years 2009–2022, an increase of 8% SOC in this size-fraction of WSAm<sub>a</sub> was observed. The dynamics of SOC in other size-fractions of WSA for G treatment were in a wide range for an investigated time. At the same time, the changes of  $C_L$  in WSA were relatively in wide range in all treatments for a period of 14 years.

### Relationships between soil organic matter and water-stable aggregates

SOM is an important factor influencing the formation of soil aggregates because it binds soil particles into aggregates and maintains their stability (Tisdall and Oades 1982; Six et al. 2000; Bronick and Lal 2005; Šimanský et al. 2022). Overall, the SOM content is higher in the macro than in the microaggregates (Tisdall and Oades 1982; Six et al. 2000). This was confirmed by the results of this study (Table 3). A clear difference can be observed between large (> 2 mm) and small aggregates (< 2 mm). PC1 was strongly positively correlated with the content of large WSAm<sub>a</sub>, while PC2 with the content of small WSAm<sub>a</sub> and WSAm<sub>i</sub> (Fig. 6). It means that large WSAm<sub>a</sub> and the SOC and  $C_L$  contained in them are more sensitive in time compared to small aggregates, which are more stable. As reported Šimanský et al. (2016) in generally as

well as Veloso et al. (2020) for vineyard soils of Slovakia, a higher SOC content increases the stability of aggregates, which is reflected in a better structure, but the stability of organic matter incorporating the soil also plays a key role. Stability of SOM in WSAm<sub>i</sub> can be linked with particle-size distribution in individual size-fraction of WSA. In WSAm<sub>i</sub> are dominated by clay- and silt-sized minerals that usually contain high proportions of sorptively preserved SOM or chemical factors (Six et al. 2004). In addition, the SOM in them can be stabilized through occlusion into soil aggregates or condensation of recalcitrant C compounds (Six et al. 2000).

In the case of this study, higher SOM content was found in WSAm<sub>a</sub> than in WSAm<sub>i</sub> in all treatments (Table 3). However, PCA showed that both SOC and  $C_L$  in large WSAm<sub>a</sub> > 2 mm were strongly negatively correlated with WSA content, indicating that although SOM content is higher in WSAm<sub>a</sub> than WSAm<sub>i</sub>, it is more sensitive to changes. Higher inputs through poultry manure (T + M) or through above-ground or root biomass (G) and biomass increase as a result of higher rates of nutrient inputs (NPK 2) can increase the organic matter input to the soil, but in some cases also slow down decay rates (for example in NPK 1) help to explain the increased SOC. WSAm<sub>a</sub> have higher SOC and  $C_L$  contents because of the conversion of organic-matter-binding microaggregates into macroaggregates and because this organic matter is qualitatively more labile and less highly processed (Table 1) than the organics stabilizing microaggregates (Six et al. 2000). Accumulation of particulate organic matter and deposition of microbial by products between microaggregates become important mechanisms in further stabilizing macroaggregates and result in an increase in SOM. For this reason, significant reservoir of SOM is individual size-fractions of water-stable aggregates in the soil (Table 3). However, the relationships between SOM in soil bulk and individual WSA size-fractions differed (Table 4) as compared to the relationships between SOM in WSA and WSA. Between SOC in bulk soil and large size-fraction of WSAm<sub>a</sub>, a stronger correlation was observed compared to SOC in soil bulk vs. small WSAm<sub>a</sub> < 2 mm and WSAm<sub>i</sub>. In the case of  $C_L$ , a large variability was observed depending on the treatment. Correlations were weakest when all treatments were included in the same analyzed set and more significant when considered individually depending on soil management. This supports hypothesis (H 4) that SOC will have a more significant influence on the content of water-stable aggregates than labile carbon. It was also shown that soil management practices in the vineyard significantly affects the aggregation (content of individual size-fractions of WSA) depending on the change of SOC and  $C_L$  in soil bulk.

## Conclusion

The built-up of SOM into the soil bulk and size-fractions of water-stable aggregates are depended on the form of SOM, and in the case of water-stable aggregates, also on their individual size-fraction. Overall, this process is affected by soil management practices in vineyard. The SOM in the soil increased most intensively due to the application of lower rates of mineral fertilization. In water-stable aggregates, soil organic carbon increased more than labile carbon across all soil management practices. Soil organic carbon was built-up most intensively in water-stable macroaggregates in size-fractions of 5–2 mm and water-stable microaggregates after the incorporation of poultry manure, which resulted in an increase in SOM, but overall, did not provide any improvement in soil structure. From the viewpoint of SOM and soil structure, the best soil management appeared to be the application of NPK at lower rates to grass strips. However, from the point of view of the improving soil erodibility using vegetation for viticulture, only grass strips between vine rows most effectively improved soil structure and C sequestration.

Soil organic carbon had a more significant influence on the content of water-stable aggregates than labile carbon. Intense changes in SOM took place in larger size fractions of water-stable macroaggregates above 2 mm, while smaller water-stable macroaggregates below 2 mm and water-stable microaggregates were more stable. Based on this, we recommend that winegrowers pay attention to proper soil management in vineyard, which affects the quality of organic matter and its transformation processes in the soil, including its stabilization, especially in WSAm above 2 mm.

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## Declarations

**Conflicts of Interest** The authors declare no conflict of interest.

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