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Evaluation of soil microbiology and soil life in different viticulture cultivation techniques

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USED ABBREVIATIONS

AMF: arbuscular mycorrhizal fungi

CEC: cation exchange capacity

C : carbon

DHA: dehydrogenase activity

FT: fresh plantation (2 years) + conventional tillage

FM: fresh plantation (1year) + conventional tillage +organic manure

pH : potential of hydrogen

OT: old vineyards + conventional tillage

OC: old vineyards + cover crop between the rows

SOC: soil organic carbon

SOM: soil organic matter

1. INTRODUCTION AND OBJECTIVES

Vine farming is a significant economic sector of global agriculture and has a long cultural history in many parts of the world (Tilman et al., 2002). However, in a number of vineyards, ecosystem services are particularly at risk because agricultural practices that have been used ineffectively and harmfully for a long time to manage weeds and pests frequently compromise the function of the soil.The great advances in agricultural productivity over the past half-century have unfortunately been accompanied by the heavy use of chemical fertilizers, irrigation water, agricultural machinery, and pesticides (Pretty, 2008). This intensification of farming systems has come at the expense of respect for the environment. Since then, there has been a loss of soil fertility and organic carbon, soil erosion, groundwater and surface water pollution,depletion, and loss of biodiversity (Bender, 2016), all of which can result in a decline in vine quality and quantity. For instance, long-term abuse of synthetic fertilizers poses a grave and dangerous threat to the environment of vineyards because it alters the context of humic minerals and microbes.

These serious problems clearly show that conventional agriculture is no longer adapted to feed humans and preserve ecosystems in the long term. Other, more environmentally friendly alternatives are being developed to address the fundamental problems of food production in an environmentally sound manner. Conservation agriculture is one such alternative (Meynard, 2008). While conventional agriculture is driven solely by productivity and profit, conservation agriculture integrates environmental, biological, chemical, and physical sciences, in a global way to develop new agricultural practices that do not degrade the environment.

In vineyards in Hungary, the exclusive use of mineral fertilizers, along with soil tillage, usually leads to a loss of soil organic matter (SOM) and reduction of soil water holding capacity and soil structural stability, resulting in a decline of soil quality and environmental degradation. Cover crops and organic fertilization are conservation management practices that can provoke changes in soil quality which requires evaluation. In this context, the objective of our work was to compare vineyards: (1) of different age: fresh plantations and old vineyards, (2) with different soil management: mechanical, covering crops, and (3) with different cultivation method: conventional, organic and to study their effect on the most important biological and chemical parameters of the soil such as: humus content, humus quality, humus stability, dehydrogenase activity, glomaline, labile carbone, pH and EC. Thus, this review aims to highlight new agronomic techniques capable of enhancing the resilience of the system and contributing to conservation and ecosystem services provision.

2. LITERATURE REVIEW

2.1. Soil management in vineyards

Soil management in vineyards is critical not only for grape productivity and quality, both in conventionall and biological management,but also for greater sustainability of the production. Conservational soil management techniques play an important role, compared to conventional tillage, in order to preserve biodiversity, to save soil fertility, and to keep vegetative-productive balance (Corneo et al., 2013).

Because of the poor stability of soil aggregates with poor organic matter content, erosion has severely damaged many viticultural soils. The rise of organic farming today attach great importance to the organic component of the soil. Increasing biological interactions in the soil to enhance its physical, chemical, and biological properties is one of the main goals of organic farming. Wine quality and specificity are greatly influenced by the terroir of the vineyard and, in particular, the soil and its microbial community. Interest in organic viticulture and interest in converting conventional viticulture systems to organic viticulture systems has increased due to concerns about the quality of food and the environmental effects of cultivation techniques (Fritz et al., 2020). Numerous studies have demonstrated that organic farming significantly impacts soil quality and biological activity more than conventional farming (Carpenter-Boggs et al., 2000; Fliessbach et al., 2000; Shannon et al., 2002).

Soil management strategy (e.g., no till farming) has been shown to prevent loss and increase soil organic matter (SOM) content in agricultural soils (Montgomery, 2007; Blanco-Canqui and Lal, 2011). Irrigation, conversion of cultivated land to pastures, introduction of earthworms, changes in grazing management, fertilization, planting grasses and legumes as cover crops (Conant et al., 2001), conservation tillage, and crop residue incorporation are examples of agricultural practices that boost SOM content (Conant et al., 2001; Reeves, 1997; Dikgwatlhe et al., 2014).

The effect of agricultural management systems on soil fertility is generally studied with plants undergoing rotation, but less is known about soils used for perennial plants, such as the grapevine. A minimal data set of soil characteristics, such as texture, organic matter, pH, bulk density, and rooting depth, can be used to evaluate the quality of the soil.

2.1.1. Tillage

In the vineyard, tillage is carried out to maintain the soil loosening, preserve the humus and nutrients in the soil, get the chemical and biological processes going, and, last but not least, to keep the weeds under control (García-Díaz et al., 2018).

The mechanical maintenance of the ground responds to various problems frequently encountered on the vineyards:

- Mechanical control of weeds to limit water and nitrogen competition that may be caused by weeds or sown plant cover.
- Loosen the soil to improve aeration and microbial life.
- Superficially bury the amendments and fertilizers to optimize their availability and efficiency (reduce the risk of leaching, promote the degradation of organic matter, etc.).

- Loosen soil sensitive to compaction by working deeper (about 20 cm).
- Maintain flat ground between the rows.

Despite the interest in using tillage as an alternative to herbicides, there are a number of drawbacks, including a decrease in bearing capacity, increase in erosion risk compared to grassy soil, destruction of the vine's superficial roots, increase in consumption of agricultural diesel, and acceleration of the mineralization process (loss of carbon).

Tillage practices have an effect on the soil's physical and chemical properties, which in turn affect the soil organisms that live there. Tillage techniques alter the soil's water content, temperature, aeration, and degree of crop residue mixing (Kovács et al., 2022). These changes in the physical environment and the food supply of the organisms impact different groups of organisms in different ways. Understanding how management affects soil ecology is one of the challenges of this field of study (García-Díaz et al., 2018).

2.1.2. Cover Crop

Any plant species that is typically grown in a mix between vine rows (alleys) is referred to as a cover crop. Planting cover crops has become widespread practice in California vineyards, and interest in their use has grown exponentially during the past 15 years. Cover crops are a tool to help grape growers manage their soils: they protect the soil from erosion and crusting; regulate vine growth; improve soil fertility; improve soil structure, aggregate stability, and water-holding capacity; enhance biological diversity in the root zone; provide habitat for beneficial generalist predator and parasitoid insects and arachnids; and provide firm footing for harvest and cultural operations (Bugg et al., 1996, Ingels et al., 1998, McGourty 1994).

From a soil-management perspective, cover crops are a relatively inexpensive method of augmenting carbon into the soil, resulting in benefits both to the vineyard and its vines and to the environment. Leguminous cover crops can also be used to provide nitrogen (N). Frequently, compost is applied as a soil amendment and fertilizer for cover crops. This practice not only enhances their growth but also increases SOM and potentially improves soil health and quality. A diverse microflora in the rhizosphere appears to improve vine root health (Scow and Werner 1998, Lotter et al., 1999, McGourty et al., 2002).

According to Magrama (2015) there are 10,496 ha of vineyards in Madrid region. Thus, an average of 11,125 Mg of carbon (40,792 Mg of equivalent CO_2) would be annually sequestered just in vineyard soils during the first years of establishment if all vineyards in Madrid were managed with spontaneous vegetation.

2.1.3. Organic Fertilization

Fertilization is one of the most essential practices in crop production. It aims to replace nutrients in the soil. A dosage that is too generous or too parsimonious can disrupt the vines' development.

In a long-term experiment carried out by Morlat and Gravier (2008) in Chinon vineyard (France) during 23 years, the effects of several organic amendments were studied on soil, vine, grapes and wine. The results of the soil analyzes showed that the addition of manure or compost in very high doses seems to be not recommended, as they are harmful for the vine, the quality of the harvest and the characteristics of the wine. Yet, they generate the strongest soil microbial biomass. On the other hand, the practice of annual shredding of pruning wood, subject to a good sanitary condition of the vines, turns out to be an excellent agronomic practice for maintaining the rate of organic soil, without negative consequences on the vine, the quality of the harvest and the wines.

Another study carried out by Chaussod (1986), showed that repeated addition of certain organic amendments can significantly increase stocks of labile organic matter in the surface layers of the soil, with repercussions on the power soil nitrogen supply.

The impact of organic amendments on agronomic and soil quality indicators in the table grape Thompson seedless cv. was assessed by Cataldo et al. (2012). In a pot experiment with Inceptisol soil, various treatments, such as compost made from grape pomace, humic extract, microbial inoculant, and chemical NPK fertilization, were used. The results reveal that plants treated with compost and microbial inoculants developed their roots more robustly. Additionally, the mineralization of organic matter increased the availability of nutrients. All treatments that received compost showed a rise in enzymatic activities, particularly β-glucosidase, acid phosphatase, and alkaline phosphatase.

2.1.4. Weed Control

Up to 20 weed species may be present in typical vines, with three or four dominating in terms of plant population and land area covered. Different weed species are dispersed in "patchy" ways in a grapevine. There will be few or no weeds in some places, while others will have a lot of weeds. Densely populated patches can be found anywhere in the vineyard but especially along vineyard edges where conditions are favorable for the growth and perpetuation of weeds. In recent years, two herbicides, simazine and diuron, have been used to control annual weeds in vineyards. Even though effective overall control can be achieved without endangering the vines, chemicals are typically only applied for control in the vine row. The general spraying technique is practiced in some limited vineyard areas today (Winkler, 1974). In order to prevent damage to vines, some growers prefer to manage weeds without herbicides for up to two years after planting. This commonly necessitates hoeing, cultivating, or using weed knives several times during the spring and summer. Between the rows, discs or mowers can be employed. Five field studies were carried out in four Portuguese wine-growing regions in 1997 and 1998 to assess the efficacy of chemical weed control in vineyards in Mediterranean climates using either lower residual herbicide doses or only foliar herbicides. The herbicides were applied during late winter. The results indicated that proper control was achieved by the application of foliar herbicides alone or by reduced rates of a mixture of residual herbicides with foliar herbicides for at least 2 months. Three months after application, the efficacy of post-emergence herbicides and lower rates of residual herbicides decreased significantly in clay soils and under heavy rainfall conditions (Monteiro and Moreira, 2004).

2.2. Physical indicators of the soil quality

2.2.1. Soil structure and texture

One of the most important factors influencing crop production is soil structure because it determines the depth at which roots can penetrate, the water holding capacity, and the movement of air, water, and soil life (Hermavan and Cameron, 1993). For the purpose of promoting water infiltration and preventing erosion, a stable surface soil structure is crucial (White, 2010).

Texture is a crucial soil physical property for characterizing soils and has been used as an indicator in numerous studies (Larson and Pierce, 1991, Carter et al., 1997, Grace and Weier, 2007, Pattison et al., 2008). However, it is not a dynamic property, and management strategies probably won't alter soil texture over time. As a result, the indicator of soil texture is suitable for initial site characterization.

2.2.2. Soil humidity

A basic hydro physical characteristic of soil is water retention which can be described as dependence between soil water content and soil water potential. Excessive soil water results in insufficient oxygen for proper root function. Water scarcity, on the other hand, limits growth and photosynthesis and, if severe, can lead to xylem failure and canopy collapse. In general, good vineyard soil has been characterized by the following traits (Cass and Maschmedt, 1998):

- >500 mm infiltration rate per day
- >150 mm total available water in the root zone (water extracted by roots between <–0.15 MPa)
- >75 mm readily available water in the root zone (water extracted by roots <–0.2 MPa)
- >15% air-filled pore space
- <1 MPa penetration resistance at field capacity (or 3 MPa at wilting point)
- <1 d soil saturation per irrigation cycle or rainfall occurrence

Water availability strongly affects soil microbial activity, community composition and consequently, soil enzymatic activities. As soils dry, the water potential increases, and as well microbial activity as intracellular enzyme activity slows down (Geisseler et al., 2011). In the case of wet soils, increased moisture could bring into soil solution soluble OM; which might be responsible for the increase of bacterial population number (Subhani et al., 2001).

2.3. Chemical indicators of the soil quality

2.3.1. Soil pH

Soil pH is one of the most significant factors determining soil fertility, it influences the solubility of metal ions like Al, Mn, Fe, Cu, Zn, and Mo, the supply of nutrient cations and anions, and the presence and activity of soil microbes. The most suitable soil pH in terms of vine cultivation is neutral. According to White (2003), the optimum pH range for vine growth is 5.5–8. Vineyard soils that are neutral or slightly acidic generally have better nutrient balance for plant growth. Soil pH value is often a natural property of the soil and comes from the pH reaction of the parent substrate in which the soil was formed (Olivier et al., 2013).

2.3.2. Cation exchange capacity (CEC)

CEC denotes the negative charge per unit mass of soil and is calculated as the total number of moles of charge (such as Ca^{2+}, Mg^{2+}, Na^{+}, and K^{+}) that an extracting solution can displace per unit mass of soil (Heil and Sposito, 1997, Rengasamy and Churchman, 2001, White, 2003). In acidic soils, Al^{3+} and sometimes Mn^{2+} are required (Rengasamy and Churchman, 2001).

The CEC is a single value that does not identify the dominant cations, which may be important if there are worries that the concentrations of Na+ and K+ in the soil will increase as a result of irrigation source water or practices like using grape marc as compost. No viticulture-specific CEC values could be located, despite the fact that there are optimal CEC value ranges that can be used as general guidelines.

2.3.3. Soil organic carbon

Soil quality is generally determined by the quantity and quality of soil organic matter (SOM). SOM plays a key role in soil fertility and productivity through its effects on physicochemical and biological soil properties (Szombathová, 1999; Balashov and Buchkina, 2011; Gaida et al., 2013; Kotroczó et al., 2020; Juhos et al., 2021). Soil organic carbon is usually recognized as a quantitative characteristic of SOM and its content depends on soil type and texture, soil use and management etc. (Šimanský et al., 2008; Devine et al., 2014; Rabbi et al., 2014, Polláková et al., 2018).

Agricultural farming practices significantly influence soil organic carbon levels. Continuous soil tillage and incorporating organic residues or fertilization can alter soil properties such as microbial biomass, aggregate stability, and enzyme activity (Morugan-Coronado et al., 2019). Other studies showed that CS (spontaneous vegetation cover) increased soil carbon in both stable and labile fractions. These results agree with Rodrigues de Oliveira et al. (2016) results. The importance of this result is that GC (ground cover) promotes atmospheric CO_2 sequestration in the soil via increasing SOC (soil organic carbon) stocks (Stockmann et al., 2013). At the same time soil quality and fertility are improved (Gregorich et al., 1997) and erodibility is reduced (ParrasAlcántara et al., 2016).

a. Humus

More stable part of SOM is humus. Humus represents a complicated and dynamic complex of organic components which are a result of decomposition and humification of organic substances in soils (Stevenson, 1994; Brady and Weill, 1999; Szombathová, 1999).

In an equilibrium ecosystem, it is at the level of the humus that the fallout of matter incorporates the soil (foliar residues fragmented by soil organisms, root supply directly incorporated into the soil at the level of the organo-mineral horizons). This "fresh" organic matter of vegetable origin will undergo, under the action of organisms of the ground, a certain number of transformations by biodegradation and humification and give a type of organic matter specific to the considered environment.

b. Labile Organic Carbon

Labile organic matter in soil is mainly generated by the decomposition of plant and animal biomass, root exudates, and dead microbial biomass (Bolan et al., 2011). According to Chantigny (2003) and Haynes (2005), labile carbon is the SOC pool that is directly accessible for microbial activity and serves as the main source of energy for microorganisms.

The addition of organic matter as fertilizer (Gattinger et al., 2012) and reduced tillage will likely increase labile organic carbon (Cooper et al., 2016). Furthermore, these practices have the potential to improve soil aggregation, which is one of the primary processes for storing organic carbon in soil, as well as carbon and nitrogen cycling (Panettieri et al., 2015). Therefore, labile carbon has the potential to be used as an indicator of soil functions, particularly for nutrient cycling, the formation of soil aggregates, the sequestration of carbon, and the provision of habitat for biological diversity (currently measured by biological indicators such as microbial biomass and abundance of faunal groups).

2.4. Biological indicators of the soil quality

Numerous studies have examined the effects of various crop practices on soil quality, but little is known about how soil microorganisms react to the management strategies used for perennial crops like vineyards. Wine quality and specificity are greatly influenced by the terroir of the vineyard and, in particular, the soil and its microbial community.

Olea europaea L. crop management's effects were assessed by Caravaca et al. in 2002, and changes in biochemical parameters were recorded. These authors concluded that the use of agrochemicals and tillage, which includes soil removal by agricultural machinery, reduces microbial biomass and enzyme activities in soils treated with compost. Chaer et al. (2009) found that a shift from an undisturbed forest into a long-term cultivation was associated with establishing a less functionally stable microbial community.

2.4.1. Soil enzymes

Another proximal driver of soil function is soil enzyme activity, which affects nutrient availability, organic matter transformations, and biogeochemical cycling. According to numerous studies (Aon et al., 2001; Badiane et al., 2001; Vepsalainen et al., 2001), soil enzyme activities are also widely recognized as sensitive indicators of soil health and potential "sensors" of changes in soil management, soil health, microbial activity patterns, soil ecological stress, and soil fertility. Furthermore, being synthesized by microorganisms, roots and soil micro- and meso-fauna (e.g., earthworms, nematodes), enzymatic activity encapsulates complex information in a simple and informative manner.

The soil enzymatic activity correlates to labile organic C rather than to SOC (Shao et al., 2015). According to recent interpretations of the composition of soil organic matter (Stockman et al., 2013), the correlation of SOC to DHA shows that a significant portion of the soil organic matter consisted of easily decomposable organic material.

a. Dehydrogenase activity (DHA)

Dehydrogenase is an oxidoreductase found only in viable cells and is considered to be a sensitive indicator of soil quality (Madejon et al., 2007). According to Garcia-Orenes et al. (2016), soil dehydrogenases activity (DHA) is used as a measure of the activity of soil microorganisms. It transfers hydrogen from organic substrates to inorganic acceptors, which is a crucial step in the biological oxidation of soil organi c matter (SOM) (Zhang et al., 2010). Based on the literature, higher DHA levels were found in the soils under natural grass than in the vineyard soils managed conventionally and organically (by 2.3 and 2.8 times, respectively). In Island, DHA was higher in two cultivated soils (3.8 and 3.4 g g-1 h-1) by 1.2 and 1.3 times than it was in uncultivated soil (4.4 g g-1 h-1) (Guicharnaud et al., 2010).

Likewise, Błońska et al. (2017) found that DHA was 4 to 7 times lower in tilled soil than in soils under natural vegetation. This is consistent with Steenwerth and Belina (2008) who showed that cover crops added soil organic matter to the soil and improved microbial activity in the vineyard soils in California. Moreover, dehydrogenase activity was strongly correlated with soil organic C content (Madejon et al., 2007). Organic C in farmyard manure-amended soils may contribute to higher dehydrogenase activity levels..

2.4.2. Glomalin

Glomalin-related soil proteins (GRSP) are glycoproteins that arbuscular mycorrhizal fungi (AMF) produce in large amounts in soil and roots. They can be a significant component of soil organic matter, which effectively acts to bind mineral particles together, so improving soil structure (Sharifi et al., 2018). Different aspects of GRSP have been considered: the easily extractable fractions (EE-prefix), difficultly extractable fractions (DE-prefix and the sum of the two is the total glomalin content (no prefix). The portion of glomalin eliminated during the initial extraction cycle is referred to as easily extractable.

AM fungi produce glomalin, so factors that regulate AMF growth may indirectly affect glomalin stocks in soil. AM fungi are most prevalent globally, (Treseder and Cross, 2006). This pattern supports the idea that the absolute amounts of C available to AM fungi should depend on photosynthetic rates (Harris and Paul, 1987; Harris et al., 1985; Johnson et al., 2002). Glomalin levels in soils decreased due to the long-term use of fungicides on grasslands, which reduced mycorrhizal diversity and colonization (Rillig, 2004). The mycorrhizal hyphal network is severely disrupted by soil tillage, which negatively impacts GRSP production and the GRSP's ability to stabilize aggregates.

Recently, Zhang et al. (2017) demonstrated that GRSP had a high carboxyl C ratio (40 percent of the total content), a relatively high aromatic hydrocarbon ratio (30 percent of the total content), and a relatively high alkyl C (20 percent of the total content). As a result, GRSP could directly promote the SOC accumulation as it retains the number of C. In conclusion, GRSPs are advantageous for the accumulation and movement of SOC.

Moreover, GRSPs can bind soil aggregates together to increase stability and can adsorb toxic metals in the soil to lessen their negative effects on plants. Mean weight diameter (MWD), a measure of the stability of the soil aggregate, was significantly and positively correlated with soil EE-GRSP and T-GRSP in a citrus orchard) (Wu et al., 2014).

2.5. Vine growing in Hungary

In the Carpathian basin, where the climate and soil are ideal for viticulture, the vine has been grown for several thousand years. During this long period, vine growing has been developing until it reached its current level. In Hungary, they grow propagation material, table grapes, and wine grape. The produced wine grape varieties are grown in the largest area, of which 72% is white wine and 25% is red wine. Only 3% remains for table grapes. The surface of vineyards is about 63 000 ha, which can be found in 22 wine regions. The biodiversity of vine is very rich in this country. Numerous native and priceless clones and varieties are being cultivated. The resistant and winter frost-resistant varieties have an essential role in the continental climate. 25% of the vineyards are on the Great Hungarian Plain, and 75% are on hills and mountains. The training and pruning system is dominated by high cordon and cane pruning. Unfortunately, there are numerous diseases that affect vine stocks, and pollute the environment through using pesticides. Hungary's viticulturists produce top-notch grapes, which are then used by oenologists to make excellent quality wine.

2.5.1. Hungary's historical wine regions

The role of soil surface relief and climate conditions are just two of the essential effects that may significantly impact the quality of grapes and wines. The grapevine is a plant that can effectively use nutrients from surface soils, below-ground soil layers, and even from the parent materials' rocks. Grapevine can withstand drought quite well because of its extensive and deep root system, which can collect water that is hardly accessible. The quality of the soil and its composition where the vine is planted can be seen in the wines that are produced (Hajdu, 2018). Around 64 962 ha are occupied by the 22 wine regions. modern vineyards have been planted in the wine regions, which are favourable not only from the aspect of economy but they also have a beautiful landscape.

2.5.2. Type of soils

Different soil types in the Carpathian Basin are suitable for viticulture. The most characteristic soil types include sandy soil and chernozem type (Kunsági wr.), loess soil (Szekszárd wr.), basalt stone (Badacsony wr., Somló wr.), lime and dolomite (Mór wr., Villány wr.), rhyolite tuff of the volcanic rocks (Eger wr.), rhyolite, andesite, dacite soil (Tokaj wr.), luvisols and cambisols (Balatonfüred-Csopak wr.) (Kozma, 1966).

2.5.3. Climate

The Carpathian Basin has a continental climate with hot, dry summers and cold winter. Mainly the Atlantic, the Oceanic and the Mediterranean climate and continental streams may influence the climate. The average rainfall is 550 mm year-1 and 300–350 mm in the vegetation period. The rainfall distribution is not favorable. Sometimes in the vegetation period there is no rain during three months. The temperature in summer varies between 30 °C and 38 °C, sometimes it reaches 40–42 °C, while in winter it may be extremely low: it may vary between -21 °C and -30 °C. Late frost in May and early frost in October is not unusual either (Mezősi and Bata, 2016).

We can identify four main climate regions based on changes in climatic patterns. The Carpathian region will experience an increase in the frequency, length, and average intensity of drought events brought on by climate change. According to meteorological estimates, the majority of changes will occur over the continental area in both summer and winter, though the distribution of projected precipitation will be asymmetrical and, in some cases, oppositional in the two seasons (a decrease in summer and an increase in winter (Bakucs, 2020).

3. MATERIALS AND METHODS

3.1. Materials

3.1.1. Experimental site description

This study was conducted in Etyek-Buda wine region which is located in Fejér County, approximately 30 km from Budapest, in western slopes of Vértes and Velencei mountains, Etyeki plateau and Váli valley (Figure 1). The elevation of the vineyards is diverse; it ranges between 160 and 300 meters. Due to the various relief of the wine region, certain miso and micro climates characterize the terroir. The climate is continental, moderate dry and warm, with an average annual temperature of 9.7-10.0°C, which is lower than the Hungarian average. Temperature in summer is not extremely high, hard winters and winter frost-caused damages are not common. Yearly rain fall is 550-600mm, the precipitation is consistent, and hail-storms are rare in this wine region. Annual amount of sunshine is 1950-2000 hours. Wind direction is north-west and the air movement is perpetual. The total area of the region is 707 ha including 30 ha new plantations. The main bedrocks of the soil are loess or loess sediment, as well as sand, sandstone, lime, dolomite, marl and loess with granite clastic. Predominant soil types are chernozem and luvisols. Peneplains are frequent in the region. The soil pH is mainly basic (Bisztray, 2015).

Figure1: Location of the wine region (source: Google Earth 2023)

3.1.2. Experimental design

A randomized complete block design was adopted, with four treatments corresponding to four different soil management systems: old vineyards with conventional soil tillage (OT), old vineyards with a cover crop between the rows (OC), 2 years fresh vineyards with conventional soil tillage (FT), and 1 year fresh vineyards with conventional soil tillage and organic manure (FM) (Figure 2). The soil under conventional tillage of the young plantations was subjected to a 50-60 cm deep plowing before planting (Table1).

Figure 2: tested sites: **A**: old vineyards with switched rows: tilled and covered; **B**: fresh plantations (2 years) with conventional tillage; **C**: fresh plantations (1year) with conventional tillage and organic manure

Table1: Characteristics of the plantations

	Year of planting	Geographical location	Spacing	Variety	Root stock	Manure
Old plantation	1987	47°26'50.8"N, 18°42'19.7"E	3,2 x 1 m (twin-vines)	Olasz rizling	5 BB	Absent
Young plantation (without organic manure)	2020	47°26'46.7"N, 18°42'16.0"E	2,4 x 1 m	Olasz rizling	Fercal	Absent
Young plantation (with organic manure)	2021	47°25'11.0"N, 18°43'54.5"E	2,4 x 1 m	Zöld veltelini	Fercal	300Kg/ha

3.1.3. Soil Sampling

Soil sampling was carried out on May 30, 2022 at depths 0-20 cm, 20-40 cm and 40-60 cm. In each plot, three soil samples were collected to constitute a representative composite sample of each plot and depth. Soil samples were taken with an Edelman type auger.

3.2. Methods

3.2.1. Humus quality

Humus quality was determined by Hagitai method. In Hargitai's two-solvent test method, 0.5% NaOH and 1% NaF extract was prepared from the soil (Hargitai, 1963). In NaOH, the real humic substances with an acidic character dissolve (fulvic acids, humic acids); while in NaF the more stable, condensed humic substances dissolve. Photometry can be performed at 4 wavelengths (400, 480, 540, 670 nm), which are averaged, or only measured at an average of 533-540 nm. The measurement at several wavelengths is justified by the fact that the molecular weight of humic substances, and thus their light absorption, varies over a very wide spectrum. The so-called Hargitai's humus quality index is the ratio of the absorbances of NaF and NaOH filtrate (Q=ENaF/ENaOH). The humus stability index can be calculated using the following formula:

$$K = ENaF / (H \times ENaOH)$$

Where, H represents the amount of humus.

3.2.2. Gravimetric water content

Gravimetric water content was determined by applying the following procedure: after collecting the soil samples from the required soil depths, samples were transported to the laboratory in closed bags that prevent evaporation. In the laboratory, a portion of moist soil (approximately 5 g) was weighted into the previously weighted box with the lid (most often we use aluminum boxes with a tight lid, but other boxes/jars that do not change their weight during the 105 °C during drying can be used as well). The open box was placed in the drying cabinet set to 105°C. Once the soil has been dried to a constant weight, the container was removed from the oven, the lid was closed (to avoid rehydration), allowed it to cool to room temperature, and then the container was weighted with its lid.

Calculation

After weighing the container and its lid, the water content (which is applied to the dried soil) can be calculated based on the wet and dry samples:

$$\theta = [(\text{g wet soil}) - (\text{g dry soil})] / (\text{g dry soil})$$

Where:

θ = gravimetric water content, as g H_2O/g dry soil

3.2.3. Dehydrogenase enzyme

Due to the light sensitivity of TTC and TPF, the entire examination must be performed under diffused light. 1 g of wet or fresh soil and 1 ml of TTC are added to the test tubes (A and B) and vortexes (Veres et al., 2013). The control tube (C) contains only 1 ml of Tris buffer (without TTC). Blank samples without soil (S) were also prepared: 1 ml of Tris buffer and 1 ml of TTC solution were added to these test tubes. The test tubes were sealed and incubated for 24 h at 30 °C. At the end of the incubation time, 4 ml of methanol was added to each test tube and shaken thoroughly, then incubated further for two hours in the dark (shake the test tubes at intervals). The soil suspension (6 ml) was centrifuged or filtered, and the clear supernatant was measured against a blank at 546 nm (red color). A calibration curve was prepared, with the help of which the final values were calculated.

Calculation

We read the TPF concentrations (µg/ml) from the calibration curve adjusted to the control and calculate as follows:

$$\text{Dehydrogenase activity (TPF µg/dry soil g)} = \text{TPF (µg/ml)} \times V/dwt \times m$$

Where:

dwt: dry mass of 1g fresh soil

m: mass of wet soil measured (g)

V: the volume of the solution added to the soil during the test (ml)

Figure 3: Determination of déhydrogenase enzyme activity

3.2.4. Labile carbon

Different proposals have been made for the standardization of soil carbon oxidizability testing with potassium permanganate (Weil et al., 2003). The purpose of these measurement methods is to estimate the "active"/"labile" carbon content of the soil - more accessible to plants and microbes - which include the carbon content stored in the soil's microbial biomass, organic matter and carbohydrate molecules. The active organic carbon content that can be determined in this way is more sensitive to the effects of soil interventions than the total organic carbon content.

Process

1g of soil sample was measured out, 2 mm (obsession 0.5 mm) sifted through a sieve, then 10 ml of potassium permanganate solution was added and the solution was shaken for 5 minutes, 125 RPM. After that, it has been centrifuged (3000RPM, 5 minutes) or filtrated. Later on, 200 µl of the supernatant was taken in a test tube and 10 ml of distilled water was added. Finally, absorbance was measured on 565 nm.

Evaluation

Record a calibration curve

To convert the measured absorption values to solution concentration, the light absorption of $KMnO_4$ solutions was determined with the following concentrations:

1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 40 mg/l

A regression line that can be fitted to the obtained values is used to convert the obtained values.

Calculation

The active carbon content is proportional to the loss of the oxidizing agent, i.e. to the fading of the purple color of the potassium permanganate, which results in a lower absorption. To calculate the numerical result, consider Blair et al.'s (1995) assumption that 1 mol of MnO4 is consumed ($Mn_7+ \rightarrow Mn_4+$ reduction) by the oxidation of 0.75 mol (9000 mg) of C:

$$\text{Active C [mg/kg]} = \text{Labile C [mg /kg]} = (0.02 – X) * 9000 * 10$$

Where:

X: standard curve mol/LC

(0.02 – X): oxidized carbon

Figure 4: Determination of soil labile carbon

3.2.5. Glomalin

The Glomalin was determined by BCA method. For the Glomalin extraction, 2 x 2g soil from the dry samples were weighted and putted in tubes. In case of TOTAL Glomalin, 8ml 50mM (pH=8) of Citrate Buffer was added to every tube. Then, falcons were caped uncompleted, and autoclaved at 121°C for 60 minutes. Subsequently, tubes have been centrifuged 5000 rpm for 15 minutes. To this end, supernatants were taken carefully without the under soil and transferred to sterile tubes and putted in in the fridge until the analysis.

For the Glomalin measurement, 20 microliter of samples was taking out and gives 1ml SWR. Tubes were shaken with vortex, and incubated for 30 min at 60°Celsius. Then, samples have been cooled to room temperature. Samples were shaken again. Spectrophotometer 562 nm was used during 1 hour. After reading data and recording them, data was putted in the function measured before by excel and the Y amounts were obtained. These amounts are values in mg per 2 g soil. So, we should divide the values on 2 for obtaining values (mg/g).

3.2.6. Soil pH

To determine the pH value, 5 g of air-dry soil was measured into a beaker and 12.5 ml of distilled water (or KCl, CaCl) was added to it. After 24 hours or 30 minutes of standstill, the glass electrode of the pH meter was placed into the solution and then the value was measured. According to the table below, we can determine the pH of our soil samples.

Table 2: Table of pH indicator ranges

Name	pH
Strongly acidic	< 4.5
Acidic	4.5 – 5.5
Weakly acidic	5.5 – 6.8
Neutral	6.8 – 7.2
Weakly alkaline	7.2 – 8.5
Alkaline	8.5 – 9.0
Strongly alkaline	> 9.0

3.2.7. Electrical Conductivity of the soil (EC)

To determine the EC, 5 g of air-dry soil was measured into a beaker and 25 ml of distilled water was added to it. After shaking for 1 hour (or putting in a shaking machine), the sample was filtered through a filter paper and EC was measured.

4. RESULTS

This part of the document will be devoted to the presentation of the results of the different treatments on the biological and chemical properties of the soil.

4.1. Soil organic matter (SOM)

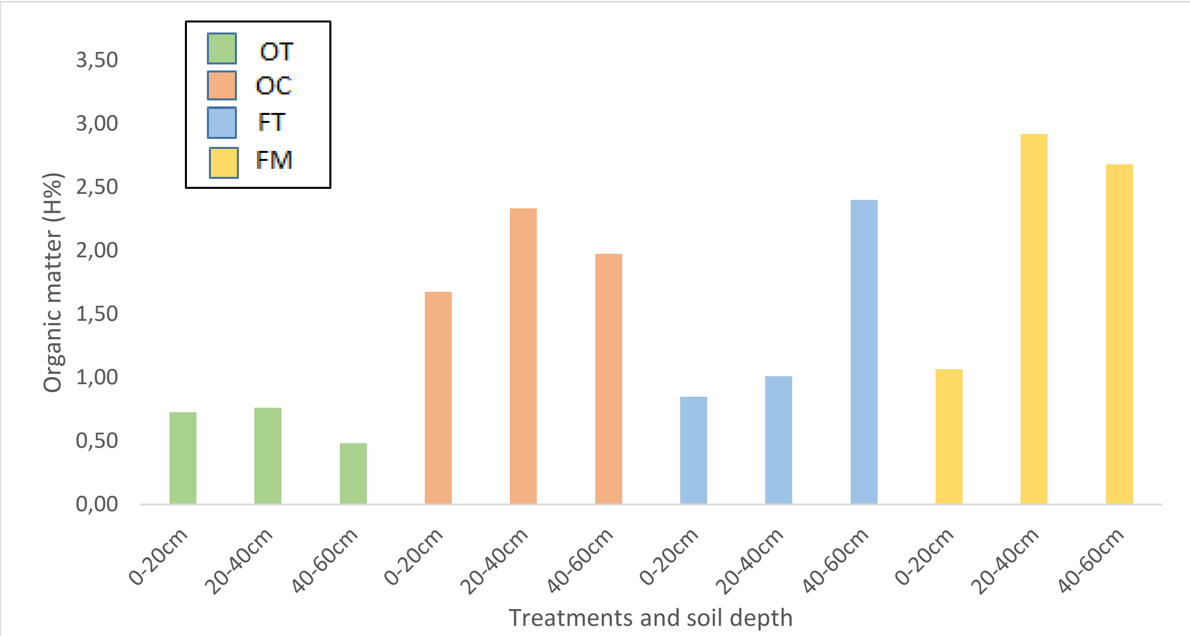

Figure 5: Organic matter in percentage at the soil depths (0-20cm), (20-40cm) and (40-60cm) for the different applied treatments; OT: old vineyards + conventional tillage, OC: old vineyards + cover crop between the rows, FT: fresh plantation (2 years) + conventional tillage, FM: fresh plantation (1year) + conventional tillage +organic manure.

According to Figure 5, we can see that at depth 20-40cm of the soil, the fresh plantation with a conventional tillage that having received organic manure recorded the highest amount of soil organic matter (SOM) (2.92%), while the lowest value (0.48%) was recorded in the old vineyards with conventional tillage at depth 40-60cm. And by comparing the absolute average for each treatment, we got the same result; old vineyards with conventional tillage had the lowest average (0.66%), and fresh plantation (1year) that have received an organic manure had the highest average (2.22%), and for the two other treatments the averages were close to each other; 1.99% for old vineyards with a cover crop and 1.42% for fresh plantation (2 years) with conventional tillage.

Regarding the soil organic matter in case of every treatment we notice that:

- In old vineyards with conventional tillage, the lowest organic matter was in the deepest sample (40-60cm), the depths 0-20cm and 20-40cm were almost the same, and the difference was only 0.03% and the lowest amount in the deepest sample was 0.2% lower than the two other depths.
- In old vineyards with a cover crop between the rows, the highest organic matter was at depth 20-40cm and the lowest value was at depth 0-20 cm, the difference between the two values is 0.66% and for the deepest sample,

the organic matter is between the two other depths, in fact its value was 0.36% lower that the humus amount at depth 20-40 cm and 0.33% higher than the humus amount at depth 0-20 cm

- In fresh plantation (2 years) with a conventional tillage, we notice that the highest amount of organic matter is at depth 40-60cm with a significant difference with the two other depths, this difference attends 1.55% in case of depth 0-20 cm and 1.39% in case of depth 20-40cm. The lowest amount was recorded in the first layer of the soil.
- In fresh plantation (1year) with conventional tillage and organic manure, the depth 0-20cm recorded the lowest value of organic matter while the highest value was at depth 20-40cm. The difference between the two is 1.85%, and for the depth 40-60cm the value was similar to depth 20-40 cm and the difference was only 0.24%.

4.2. Humus quality

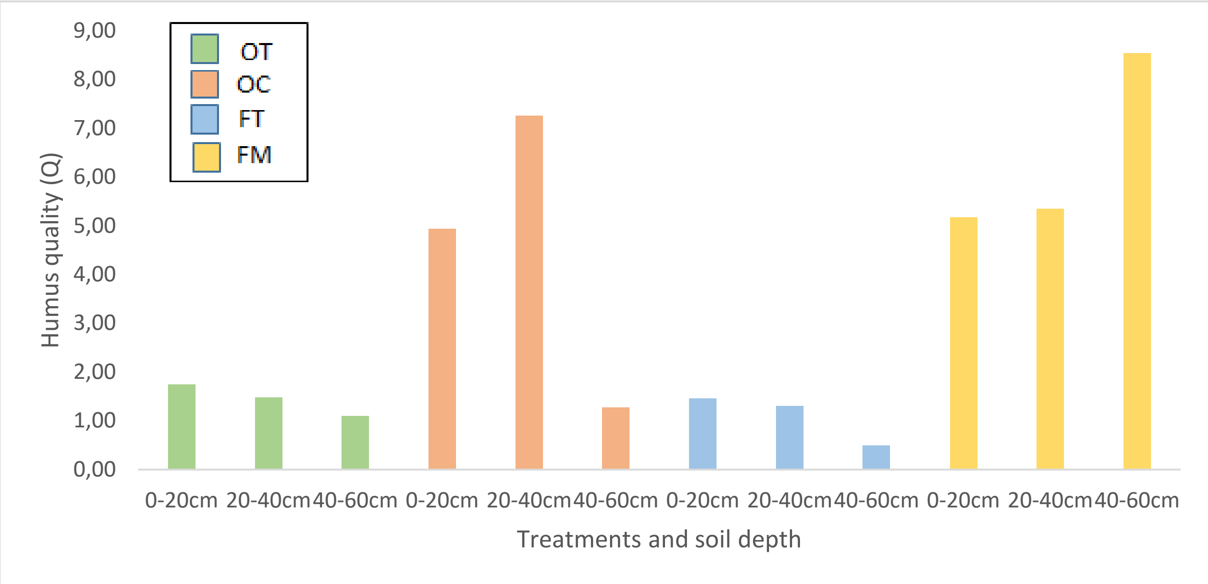

Figure 6: Humus quality (Q) at the soil depths (0-20cm), (20-40cm) and (40-60cm) for the different applied treatments; OT: old vineyards + conventional tillage, OC: old vineyards + cover crop between the rows, FT: fresh plantation (2 years) + conventional tillage, FM: fresh plantation (1year) + conventional tillage +organic manure.

The highest humus quality (8.55) was recorded in fresh plantation (1year) with conventional tillage and with organic manure at depth 40-60cm, whereas the lowest value (0.50) was in fresh plantation (2 years) with conventional tillage at depth 40-60cm (Figure 6). And by comparing the absolute average of humus quality for each treatment, a significant variation can be observed between old vineyards with conventional tillage (1.45) and old vineyards with cover crop (4.50), and also between the two fresh plantations, vineyards without organic manure recorded only 1.09 while vineyards with organic manure had much more better humus quality (6.36).

Regarding the humus quality in case of every treatment we notice that:

- In old vineyards with conventional tillage, we notice that the humus quality was almost the same at the three depths, slightly decreasing with the depth, in fact the highest value was at depth 0-20cm followed respectively by depths 20-40cm and 40-60cm. The differences between them were insignificant (0.26, 038 and 0.64).

- In old vineyards with a cover crop between the rows, the best humus quality was at depth 20-40cm with a value reaching 7.26, followed by the depth 0-20cm (4.94) and the lowest humus quality (1.28) was recorded at depth 40-60cm. As we can see the difference between the highest and lowest value is very significant (5.98).
- In fresh plantation (2 years) with a conventional tillage, the lowest value was at the deepest sample, the depths 0-20cm and 20-40cm were almost the same, and the difference was only 0.15 and the lowest amount in the deepest sample was 0.95 lower than the two other depths.
- In fresh plantation (1year) with conventional tillage and organic manure, we recorded a very good humus quality in the three depths. The highest value was at depth 40-60cm, the depths 0-20cm and 20-40cm were almost the same, and the difference was only 0.17 and the highest amount in the deepest sample was 3.20 higher than the two other depths.

4.3. Humus stability

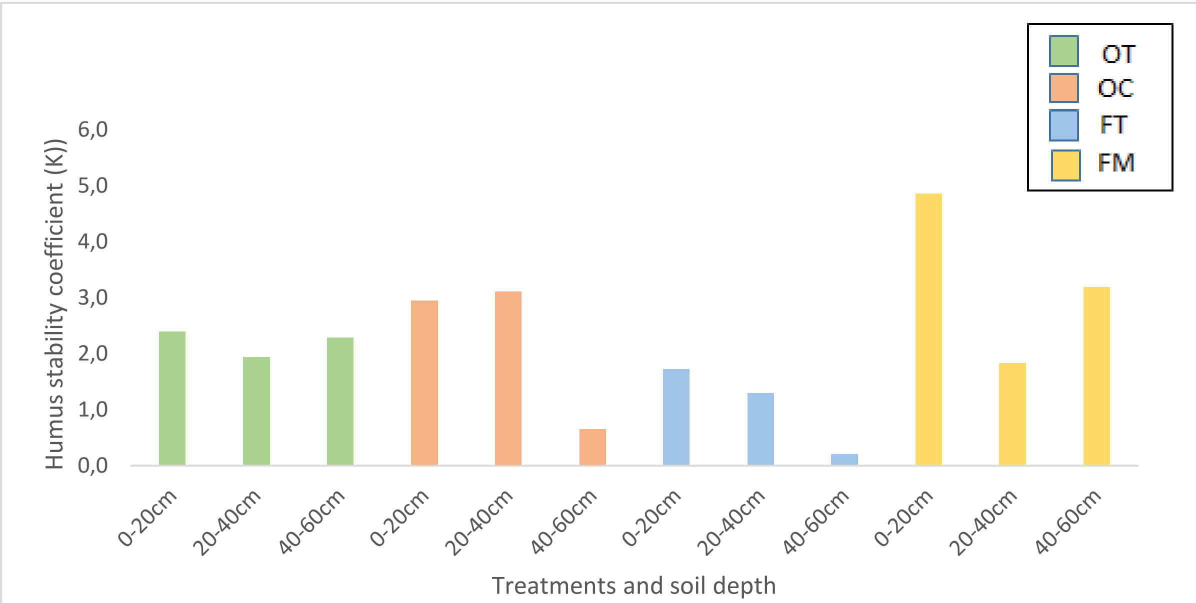

Figure 7: Humus stability (K) at the soil depths (0-20cm), (20-40cm) and (40-60cm) for the different applied treatments; OT: old vineyards + conventional tillage, OC: old vineyards + cover crop between the rows, FT: fresh plantation (2 years) + conventional tillage, FM: fresh plantation (1year) + conventional tillage +organic manure.

According to Figure 7, we notice that at depth 0-20cm of the soil, the fresh plantation (1year) with a conventional tillage that having received organic manure recorded the highest value of humus stability (4.9), while the lowest value (0.3) is recorded in fresh plantation (2 years) with conventional tillage at depth 40-60cm. By comparing the absolute average for each treatment, we notice that the two old vineyards, conventionally tilled and with a cover crop have the same average for humus stability (2.2), and for the two fresh plantation, there is a significant difference between them, vineyards with organic manure had more humus stability (3.3) than vineyards without organic manure which recorded the lowest humus stability (1.1).
Regarding the humus stability in case of every treatment we notice that:

- In old vineyards with conventional tillage, the humus stability was almost the same at the three depths with a slight difference. The highest value was at depth 0-20cm followed by the value at depth 40-60cm and the lowest value was at depth 20-40cm. The difference between the highest and lowest value was only 0.45.
- In old vineyards with a cover crop between the rows, the lowest humus stability was at depth 40-60cm, the depths 20-40cm and 40-60cm had almost the same value with a difference of 0.16 only, whereas, the difference between the highest value at depth 20-40cm and the lowest one reached 2.5 which is a significant difference.
- In fresh plantation (2 years) with a conventional tillage, the highest humus stability was at depth 0-20cm followed by the depth 20-40cm, the difference between the two is only 0.43, and the lowest value was at the deepest sample with a difference of 1.52 between it and the highest value.
- In fresh plantation (1year) with conventional tillage and organic manure, the highest humus stability was recorded at depth 0-20cm and the lowest value was at depth 20-40 cm, the difference between the two is 3, for depth 40-60 cm humus stability was 1.7 lower than at depth 0-20 cm and 1.3 higher than the humus stability at depth 20-40 cm.

4.4. Dehydrogenase activity

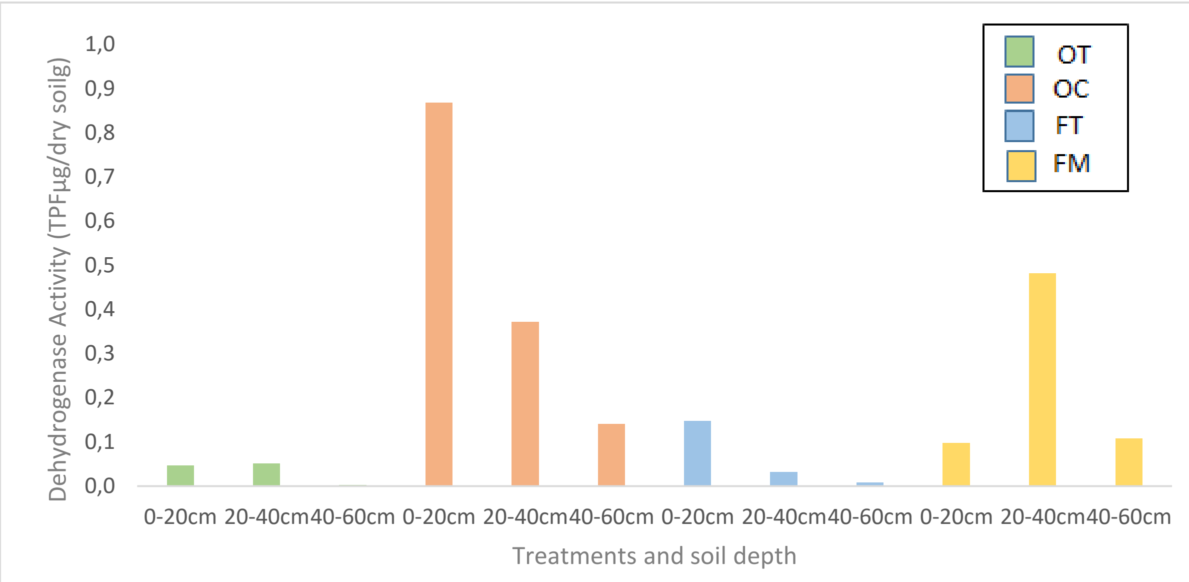

Figure 8: Dehydrogenase activity at the soil depths (0-20cm), (20-40cm) and (40-60cm) for the different applied treatments; OT: old vineyards + conventional tillage, OC: old vineyards + cover crop between the rows, FT: fresh plantation (2 years) + conventional tillage, FM: fresh plantation (1year) + conventional tillage +organic manure.

According to Figure 8, we notice a huge difference regarding the dehydrogenase activity between the treatments. The highest activity of the enzyme (0.86 TPFµg/dry soil g) was recorded in old vineyards with a cover crop between the rows at depth 0-20cm. While, in old vineyards with conventional tillage the enzyme activity was almost absent (0.002 TPFµg/dry soil g) at depth 40-60cm and we notice the same thing in the fresh plantation (2 years) with a conventional tillage where the value was negligible (0.009 TPFµg/dry soil g) at depth 40-60cm. And when we compare the absolute average for each treatment, we notice that DHA activity is significant lower in old tilled vineyards (0,033) compared to old covered vineyards (0.460) and it is significant higher in fresh amended vineyards (0.229) than in fresh unamended vineyards (0.062). Also, we have to mention that in covered vineyards, the amount of DHA which represents the diversity of soil microbial life is much more important in top soil layer, and it decreases gradually when we go deeper in the soil layers (20-40cm and 40-60cm) so we can say that in upper soil layer, the soil life is the most diverse.

Regarding the dehydrogenase activity in case of every treatment we notice that:

-In old vineyards with conventional tillage, dehydrogenase activity was very low at the two depths 0-20cm and 20-40cm and almost absent at depth 40-60cm.

- In old vineyards with a cover crop between the rows, the dehydrogenase activity is very important at the soil surface (0-20cm) where most microbial life is found, at depth 20-40cm there is still enzyme activity but less than in the layer surface with a difference of 0.5 TPFµg/dry soil g, and in the deepest layer of the soil, the enzyme activity decreases significantly with a difference of 0.73 TPFµg/dry soil g and 0.23 TPFµg/dry respectively between the depths 0-20cm and 20-40cm.

-In fresh plantation (2 years) with a conventional tillage, the dehydrogenase activity was higher at depth 0-20cm and almost negligent at the deepest layers of the soil.

- In fresh plantation (1year) with conventional tillage and organic manure, we recorded the highest activity of the enzyme at depth 20-40cm which was not the case for the other treatments, and at depths 0-20cm and 40-60cm the enzyme activity was almost the same, the difference between the highest and lowest value was significant, it attended 0.39 TPFµg/dry soil g.

4.5. Soil humidity

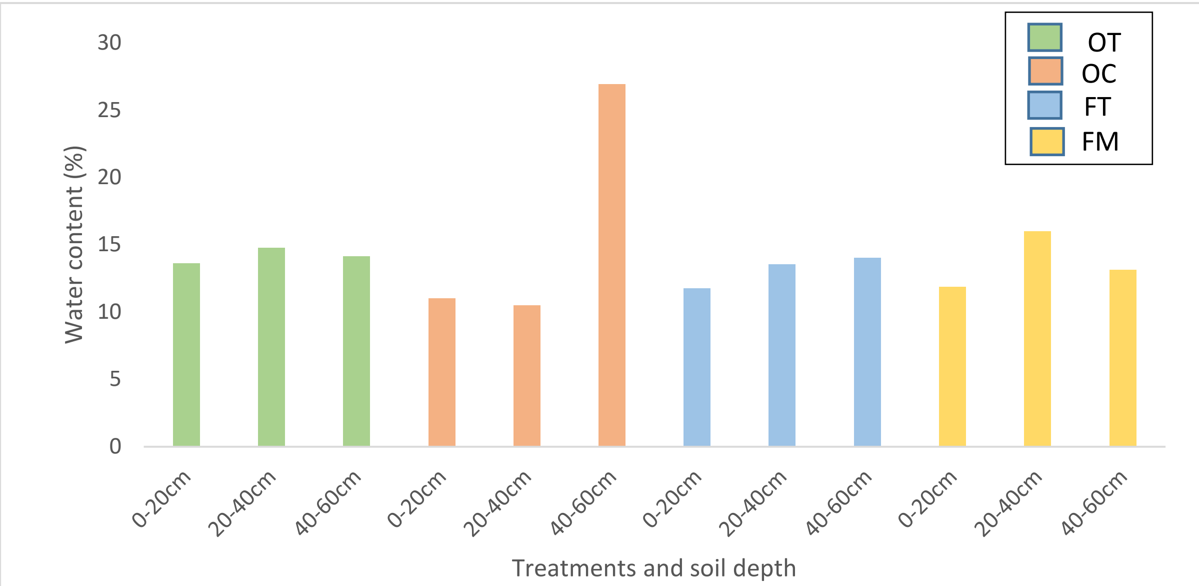

Figure 9: Soil humidity at the soil depths (0-20cm), (20-40cm) and (40-60cm) for the different applied treatments; OT: old vineyards + conventional tillage, OC: old vineyards + cover crop between the rows, FT: fresh plantation (2 years) + conventional tillage, FM: fresh plantation (1year) + conventional tillage +organic manure.

According to Figure 9, the highest soil humidity (26.9%) was in old vineyards with a cover crop between the rows at depth 40-60cm, and the lowest value (10.4%) was in the same vineyards but at depth 20-40cm. By comparing the absolute average for each treatment, we notice that soil humidity was quite similar in old tilled and in both fresh plantations (with and without organic manure), but between the old vineyards, soil humidity is a bit higher in covered (16.14%) than in tilled samples (14.17%) and for fresh plantations, we obtained almost the same average (13%).

Regarding the soil humidity in case of every treatment we notice that:

-In old vineyards with conventional tillage, soil humidity was very similar at the three depths; there is no significant difference between them. The differences were 1.17% between depths 0-20cm and 20-40; 0.64% between depths 20-40cm and 40-60cm and 0.52% between depths 0-20cm and 40-60cm.

-In old vineyards with a cover crop between the rows, soil humidity was absolutely higher in the deepest layer, the depths 0-20cm and 20-40cm were almost the same, the difference was only 0.53,% and the highest value at the depth 40-60cm was 16.44% higher than the two other depths which is significant different.

-In fresh plantation (2 years) with a conventional tillage, the values of soil humidity at the three depths were almost the same with a slight increase with the increasing of depth. The values are 11.74%, 13.52% and 14% respectively at depths 0-20cm, 20-40cm and 40-60cm.

- In fresh plantation (1year) with conventional tillage and organic manure, we recorded the highest soil humidity at depth 20-40cm, the values at the two other depths were very close, the difference is 1.27%, and the highest value was 4.13% higher than in depth 0-20cm and 2.85 %higher than 40-60cm. So, water content in middle layer seems to be significantly different compared to the two other layers.

4.6. Labile Carbon

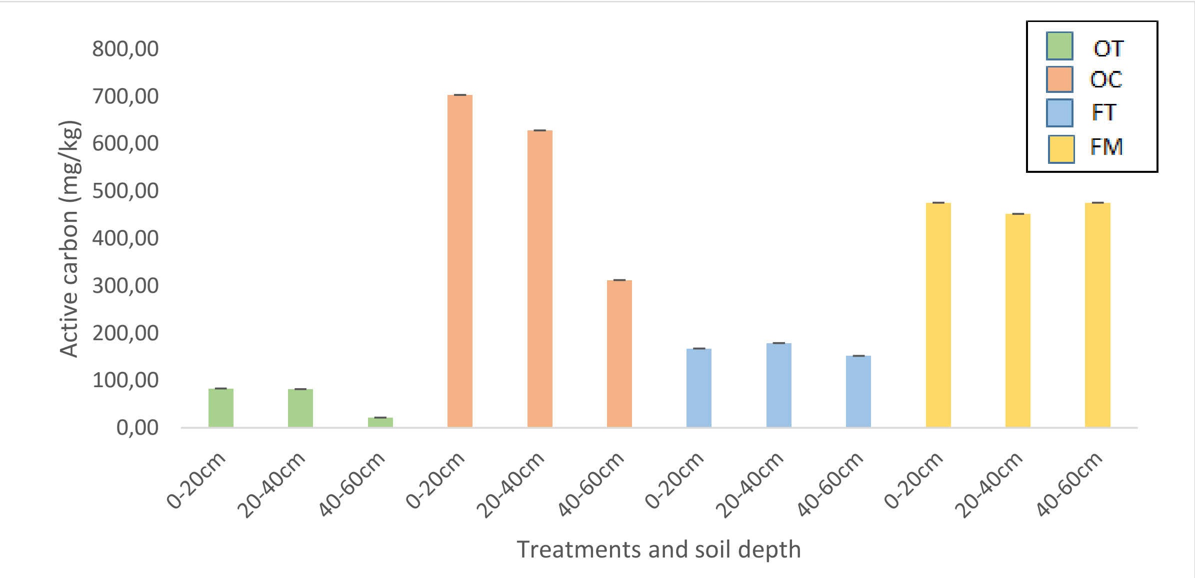

Figure 10: Labile carbon at the soil depths (0-20cm), (20-40cm) and (40-60cm) for the different applied treatments; OT: old vineyards + conventional tillage, OC: old vineyards + cover crop between the rows, FT: fresh plantation (2 years) + conventional tillage, FM: fresh plantation (1year) + conventional tillage +organic manure

Figure 10 shows that labile carbon varies enormously with the treatments, the highest amount of labile C (703 mg/kg) was recorded in the old vineyards with a cover crop between the rows at depth 0-20cm, and the lowest amount of labile-C (21.69mg/kg) was obtained in the old vineyards with conventional tillage at depth 40-60cm, we notice that there is significant difference between the two values that reached 681mg/kg. And when we compare the absolute average for each treatment, we notice that covered old vineyards had significantly greater labile C than tilled old vineyards, the average value passed from 62mg/kg to 547mg/kg. For fresh plantations, the labile C is more present in amended vineyards (467mg/kg) than in unamended vineyards (166mg/kg).

Regarding the labile carbon in case of every treatment we notice that:

- In old vineyards with conventional tillage, the lowest labile-C was in the deepest sample, the depths 0-20cm and 20-40cm were similar, and the lowest value was 60.4mg/kg lower than the two other depths.

-In old vineyards with a cover crop between the rows, the lowest labile-C was at the depth 40-60cm, the values at depths 0-20cm and 20-40cm were near to each other, and the difference between them was 74.6mg/kg, the difference between the highest and lowest value was 391mg/kg.

-In fresh plantation (2 years) with a conventional tillage, the labile-C is almost the same in the three different depths, the highest value (179mg/kg) was recorded at depth 20-40cm, the lowest one (152mg/kg) was at depth 40-60cm.

- In fresh plantation (1year) with conventional tillage and organic manure, we notice also a similarity of the labile C in the three depths, there is no significant difference between them, depths 0-20cm and 40-60cm were identical and at depth 20-40cm the labile C was 23mg/kg lower than the two other depths.

4.7. Glomalin

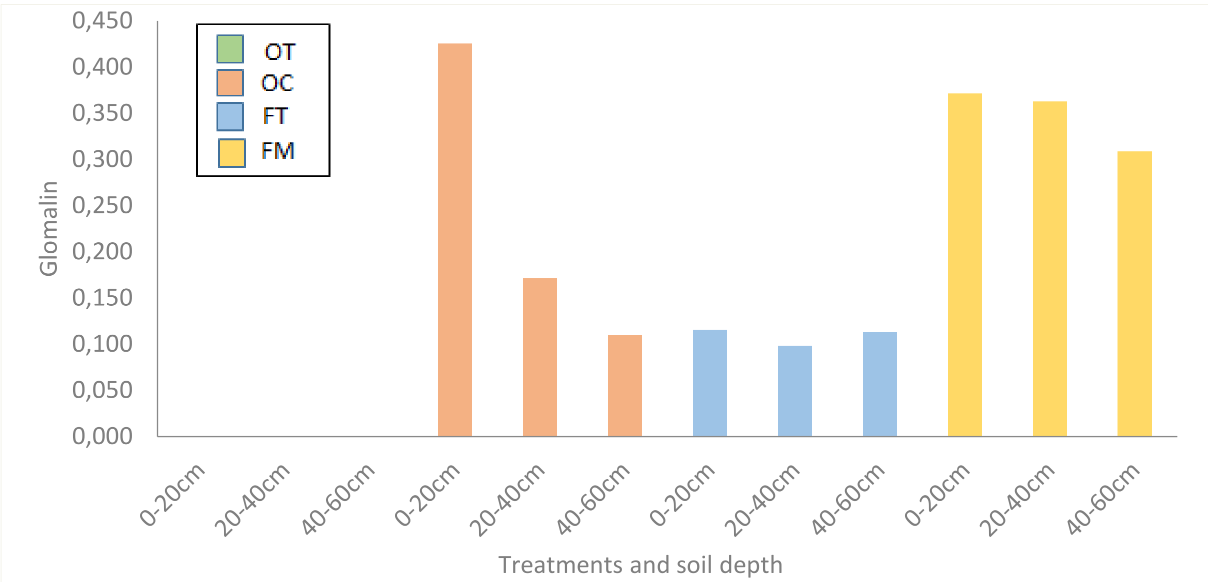

Figure 11: Glomalin at the soil depths (0-20cm), (20-40cm) and (40-60cm) for the different applied treatments; OT: old vineyards + conventional tillage, OC: old vineyards + cover crop between the rows, FT: fresh plantation (2 years) + conventional tillage, FM: fresh plantation (1year) + conventional tillage +organic manure

Figure 11 shows that, the highest amount of glomalin (0.425) was recorded in the old vineyards with a cover crop at depth 0-20cm, while glomalin was totally absent in the old vineyards with conventional tillage at the three soil layers. And when we compare the absolute average for each treatment, we notice that amended fresh plantation (1year) with conventional tillage had the highest average of glomalin (0.347), followed by the covered old vineyards (0.235) and unamended fresh plantation (2 years) with a conventional tillage (0.109).

Regarding the amount of glomalin in case of every treatment we notice that:

-In old vineyards with conventional tillage, the glomalin was absent at the three soil depths

-In old vineyards with a cover crop between the rows, the highest amount of glomalin was at the top soil layer, and it decreases gradually with the depth, the glomalin concentration passed from 0.425 to 0.171 between depths 0-20cm and 20-40cm which is a significant difference (0.254) and it reached its lowest value (0.110) at depth 40-60cm.

-In fresh plantation (2 years) with a conventional tillage, the glomalin amount was almost the same in the three soil depths, there is no significant difference between them.

- In fresh plantation (1year) with conventional tillage and organic manure, we notice also a similarity between the two first depths (0-20cm) and (20-40cm) but the glomalin amount was the lowest at the deepest depth (40-60cm).

4.8. Soil pH

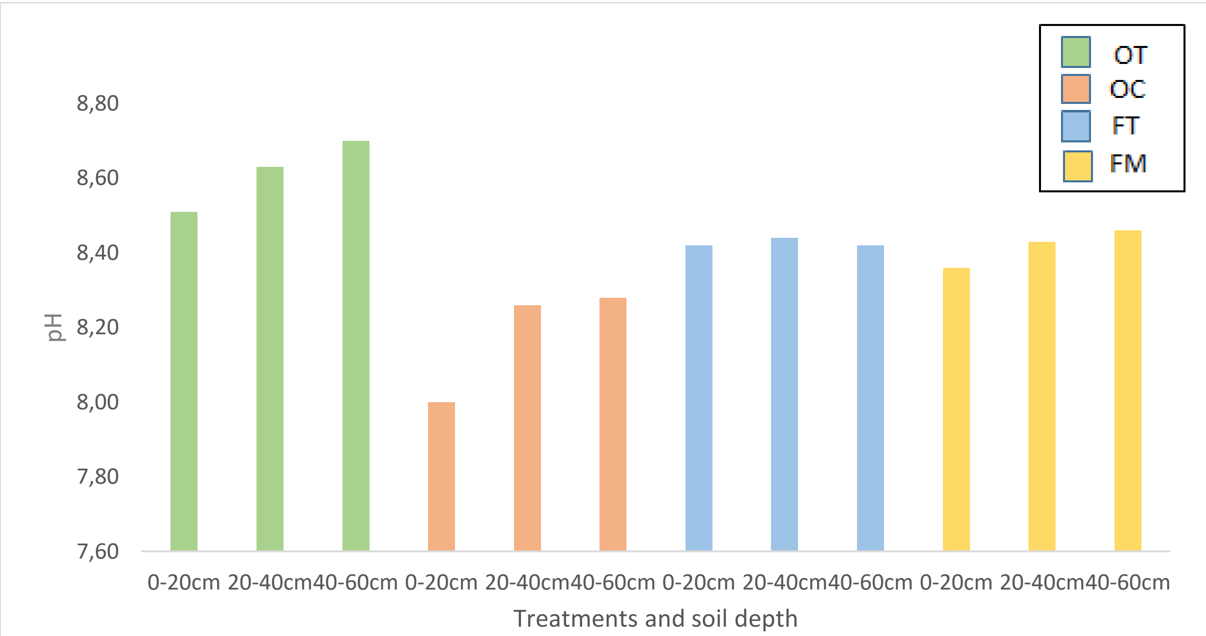

Figure 12: Soil pH at the soil depths (0-20cm), (20-40cm) and (40-60cm) for the different applied treatments; OT: old vineyards + conventional tillage, OC: old vineyards + cover crop between the rows, FT: fresh plantation (2 years) + conventional tillage, FM: fresh plantation (1year) + conventional tillage +organic manure.

According to Figure 12, the highest value of pH (8.70) was obtained in the old vineyards with conventional tillage at depth 40-60cm and the lowest pH (8) was recorded in old vineyards with a cover crop between the rows at depth 0-20cm. When we compare the absolute average for each treatment, we notice that there is a significant difference between old vineyards, the covered samples recorded a lower pH value (8.2) compared to tilled samples (8.6), but for the fresh vineyards, we got the same pH average (8.4) in amended and unamended samples, so it seems that organic manure had no significant effect on the pH.

Regarding the soil pH in case of every treatment we notice that:

- In old vineyards with conventional tillage, the pH increases with the depth, the lowest pH was at the first layer of the soil, followed by the depth 20-40cm and the highest pH was at depth 40-60cm, the difference between the highest and lowest value was 0.19.

-In old vineyards with a cover crop between the rows, the lowest value of pH was at the depth 0-20cm, the pH at depths 20-40cm and 40-60cm was almost the same, and the difference was only 0.02, and the lowest pH was 0.28 lower than the two other depths.

-In fresh plantation (2 years) with a conventional tillage, the pH was almost the same in the different depths, there is no significant difference between them, and the value is around 8.43.

- In fresh plantation (1year) with conventional tillage and organic manure, the pH is almost the same in the three soil depths, a slight difference between depth 0-20cm that recorded the lowest pH and depth 40-60cm that recorded the highest pH, the difference between the two values is only 0.1.

4.9. Electric conductivity

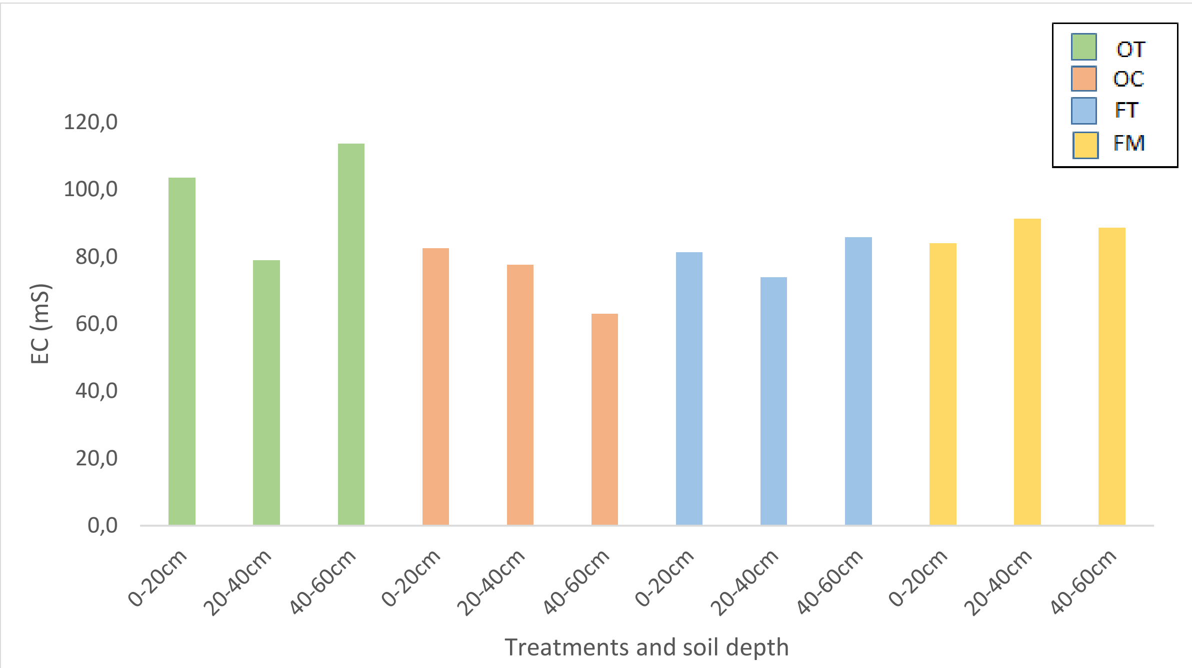

Figure 13: Electric conductivity of the soil at depths (0-20cm), (20-40cm) and (40-60cm) for the different applied treatments; OT: old vineyards + conventional tillage, OC: old vineyards + cover crop between the rows, FT: fresh plantation (2 years) + conventional tillage, FM: fresh plantation (1year) + conventional tillage +organic manure

According to Figure 13, the highest electric conductivity (113.6mS) was recorded in the old vineyards with conventional tillage at depth 40-60cm, and the lowest value (63mS) was in old vineyards with a cover crop between the rows. By comparing the absolute average for each treatment, we notice that for old vineyards the average of EC is lower in covered (74.43mS) than in tilled samples (98.70mS) and for fresh plantations, averages are close to each other, EC was respectively 80.33mS and 87.97mS in unamended and amended samples.

Regarding the electric conductivity in case of every treatment we notice that:

- In old vineyards with conventional tillage, the lowest EC was at depth 20-40cm, while the highest value was in deepest sample, the difference between the two was significant (34.7mS).

-In old vineyards with a cover crop between the rows, the lowest value of EC was at the depth 40-60cm, the EC at depths 0-20cm and 20-40cm was almost the same, and the difference was only 4.9mS, and the highest value was at the first layer of the soil, the difference between the highest and lowest value of EC was 19.6mS.

-In fresh plantation (2 years) with a conventional tillage, the EC was almost the same in the three depths with a slight difference between them, the highest value was at depth 40-60cm and it was 11.4mS higher than the EC at depth 20-40 cm and only 4.5mS higher than the EC at depth 0-20cm

- In fresh plantation (1year) with conventional tillage and organic manure, we notice also a similarity of the EC in the three depths, the lowest value was at the first layer of the soil and it is was 7.3 lower than the EC at depth 20-40cm and 4.6 lower than the EC at depth 40-60cm.

5. CONCLUSIONS

5.1. Soil organic matter (SOM)

By comparing the two old vineyards we notice that the quantity of organic matter is much more important in the vineyards with a cover crop than in the conventionally tilled vineyards, this is due to the fact that the use of cover crops results as a general rule in a remarkable increase in soil organic carbon (SOC) content and as a consequence an increase in the humus content. Also, the presence of other sources of root exudates and plant remains from the plant cover would compensate for some of the carbon lost by mineralization. The humus content in the soil is also in correlation with soil management, the intensification of agriculture has considerable influence on the balance of soil organic matter (SOM) and so on humus content. The intensity of cultivation supports the process of mineralization and decrease of organic matter in the soil, there is no replacement for this humus lost. While no-tillage system promotes carbon storage in the soil surface. As it concerns the treatments with and without organic manure, it's quite obvious that organic manure will be a direct source of SOC which will increase the humus content as well. By comparing the soil organic matter (SOM) in the different soil layers of fresh plantation (1 year) with a conventional tillage and organic manure, we notice that the top soil layer is poorer in organic matter than the deepest layers. This could be a result of the fresh mechanical practices such as plowing and turning of the soil that will put the organic matter into the deepest soil layers, and fresh plantations require strict mechanical weed control to provide the best conditions (without competitors) for the early development. Thus, the covering of organic manure (to protect it from direct sunshine) and the mechanical cultivation could explain why organic matter is much better in deepest soil layers than on its surface. Also we have to mention that these are compulsory practices for any fresh plantation during the establishment phase.

5.2. Humus quality

The data of humus quality showed that conventional tillage results in a significant decrease in soil humus quality compared to no tilled vineyards. We can explain that by the fact that the absence of soil disturbance will significantly increase the content of humic acids strongly bound with the soil clay minerals in the top soil layers and as a result increase the humus quality. Also, we noticed that in amended vineyards, humus quality was satisfactory compared to unamended vineyards. A significant increase was observed, especially at the deepest soil layer (40-60cm), as a result of the application of organic fertilization. In fact, the quality and stability of humic substances in the soil is determined by a regular introduction of a certain amount of organic matter, which determines the formation of new humic substances and their destruction due to the formation of a stable amount of active humus forms (Teit, 1991).

5.3. Humus stability

The ability of soil aggregates to resist exogenic action and remain stable when exposed to changes in the external environment is determined by their stability. According to Zheng et al. (2018), the conservation strategies of no-tillage improved soil structure and increased the number of macro-aggregates by reducing the disturbance frequency of tillage and keeping high stubble cover, which contributed prevent erosion, whereas frequent tillage weakens the clay-humus stability of soil organic matter and microbial biomass making them more prone to decay. But, the results obtained didn't show a significant difference in humus stability between the tilled and covered old vineyards unlike to what we expected. And the humus is more stable in amended vineyards than in unamended vineyards, this could due to the presence of organic manure. Supposedly, the drastic mechanical treatments (plowing) before planting effected humus stability negatively, which could be compensated a bit with the presence of organic manure.

5.4. Dehydrogenase activity

The metabolic activity of microbial community can be evaluated by determining enzyme activities (Deng et al., 2006). Dehydrogenase activity was used to evaluate microbial metabolic activity.

According to results, we notice that there is no significant soil life in mechanical cultivation in old vineyards as well as in fresh plantations without organic manure (treatments OT and FT). For fresh vineyards, this could be explained by the fact that before plantation, there was a heavy mechanical working on the soil which could result in lower intensity of soil life. Planting is a quite intensive treatment that could disturb the microbial life. And same for tilled old vineyards, tillage contributed to distinct distributions of bacterial communities. Unlike, no-till and cover crop plots will have higher microbial abundance and diversity due to more carbon and greater heterogeneity in microenvironments; which was compatible with what we found. The enhanced soil microbial biomass and activity associated to cover crops is majorly concentrated in top soil layers (0 - 20 cm), mainly as a consequence of the increase in SOC content mentioned above. In particular, it is the particulate organic matter C which most relevantly increases nutrient availability to microorganisms (Agnelli et al., 2014; Belmonte et al., 2018; García-Díaz et al., 2018; Peregrina et al., 2010; Peregrina et al., 2014).

From another perspective, organic manure had a significantly greater impact on the dehydrogenase activity when we compare amended to unamended vineyards (treatments FT and FM); this is due to the promoting effect of the organic manure on the growth of microbial community by providing nutrients to them. According to Fraser et al. (1988), the DHA has been associated with the amount of readily available organic C substrates in the soil. The higher levels of DHA may be the result of its higher organic C. Also when we compare the different soil layers of fresh plantation (1 year) with a conventional tillage and organic manure, we found that enzyme activity was low at top soil layer, this could be due to the mechanical cultivation before plantation (such as turning and deep plowing) which will push the microorganisms from the top soil layer to the deepest layers where there is less oxygen. So, this change of the living environment will result into a decreasing in the microbial activity at the soil surface.

5.5. Soil humidity

For soil humidity, we found that it is more important in plant covered than in tilled vineyards, in fact, the plant cover acts as a barrier to evaporation; increases water retention capacity and maintains residual humidity from the rainy season (Soltner, 2003). According to Chabanne et al. (1999), the use of cover crops also captures deep moisture through their roots, thus improving the water balance. This could explain why soil humidity is much more important in the deepest layer of the soil where the majority of grapevine root system is found, so there will be no competition for water between cover crop and grapevine but rather cover crop will play a role of protector and will preserve the water. Also, in a humid climate, cover crop improves water infiltration and slowing down runoff (Scopel et al., 2004). The greater ease of infiltration and drainage in the soil allows water to return to the field more quickly.

On another side, we found that soil humidity is quite similar between old tilled vineyards with tillage and the two fresh plantations (with and without organic manure); this can be explained by the fact that mechanical cultivation is typical to fresh plantations as it is the establishment phase, intensive soil management techniques were used in order to remove all plants between the rows, this is why values were similar to old plantations with mechanical tillage. As it concerns the small difference in organic manure in the middle layer found in amended fresh plantations, it could be due to the higher humus content present at this layer, and together with organic manure, they will act as a sponge and preserve the water content. This better water preservation will store a lot of water in the ground and releasing it slowly to feed the waterways and the grapevine roots.

5.6. Labile Carbon

According to the results obtained, we suggest that labile carbon fraction is sensitive to management practices such as cover crops, tillage and organic matter management.

Tillage disrupts macro- and micro-aggregates, increases soil temperature and aeration, and releases soil organic matter which was protected in these physical structures (Six et al., 2000). Soil organic matter can thus become more available to soil organisms, increasing CO_2 emissions and decreasing the labile fractions. This could explain the low labile-C found in tilled vineyards. Cover crops can act as a short-term reservoir of nutrients for crop growth in agricultural ecosystems and as a result the soil labile organic C pools will increase. We notice that OM addition increased the concentration of the labile carbon fractions, this could explain why in fresh plantations, the labile C is more present in amended vineyards than in unamended vineyards, there is a direct effect of organic matter input on labile carbon, which stimulate microbial biomass and provide a suitable physical environment for them, moreover, OM addition can introduce external microbial populations, which also can contribute to an increase of the labile organic carbon pools (Bastida et al., 2008).

5.7. Glomalin

In contrast to nearby conventionally tilled sites, the results showed that high glomalin concentrations are related to the formation and stabilization of aggregates in undisturbed and no-till systems. In fact, glomalin which is a glycoprotein produced by the hyphae and spores of arbuscular mycorrhizal fungi (AMF), was absent in old tilled

vineyards while it was well present in old covered vineyards. This could be explained by the fact that mycorrhizal hyphal network is severely disrupted by soil tillage, which negatively impacts. Glomalin-related soil proteins (GRSP) production and the GRSP's ability to stabilize aggregates. Because it directly affects the integrity of the mycelial network, tillage is known to be a significant factor in shaping AMF communities in agricultural settings (Verbruggen and Kiers, 2010).
Moreover, the glomalin concentration highly depends on the vegetation cover and the manner of soil management. An increased occurrence of glomalin was observed after the application of an organic material in fresh plantation (1year) compared to a very low concentration in unamended fresh plantation (2 years). A study by Jordan et al. (2000) revealed that cover crops could promote a different set of dominant mycorrhizal fungi, potentially providing a wider spectrum of these fungi for colonizing grapevine roots.

5.8. Soil pH

Cover crop applications had a significant decrease on soil pH in comparison with the tilled soil. The decrease in soil pH is compatible with results of Moreti et al. (2007) and Fabian (2009). As mentioned before, microbial soil life is well present in cover crop treatment, the microbes are feeding on soil humus and they produce in return CO_2 which with soil water will form the H_2CO_3 responsible for the decrease in the pH value. Cover crops could importantly affect the soil pH. Moreti et al. (2007) determined that plants have exudation of acids to the soil from their roots that could role directly on the soil pH. Besides, when organic matter is mineralized there is production of organic acids that could promote the raise of soil acidity (Garcia and Rosolem, 2010).
Regardless the pH value in the fresh plantations, we found that adding organic manure didn't affect the pH value which could be explained by the fact that soil pH gradually decrease with the increase of incubation time. The trend of soil pH reduction is about 1 unit within 60 days of incubation. In this case we should also take into consideration the source of the manure that has been used.

5.9. Soil EC

Results showed that cover crop had decreased soil electrical conductivity (EC). This demonstrated that a cover crop can at least maintain and/or enhance SOM, NO_3-N, P, C, and N (primarily in the topsoil), which can help to improve soil quality and, in turn, possibly increase grape productivity. If conductivity is a good indicator of the dynamics of soil available NO_3 levels, as suggested by Smith and Doran (1996), this may have resulted from the two-step process of organic N mineralization. In a variety of soil conditions, numerous different microorganisms contribute to the formation of the first product (NH_4). A particular class of aerobic bacteria that are more susceptible to soil temperature, soil water content, and other environmental factors are responsible for the second step of the oxidation of NH_4 to NO_3.
For the fresh plantations, we noticed that the adding of organic manure didn't have a significant effect on the soil EC, while in other studies such as the one carried by García-Orenes and al. (2016), they found that soil electrical conductivity reached higher values in the organic treatments.

6. SUMMARY

Soil management in viticulture and sustainable strategies assume greater significance to improve the quality of modern viticulture. To assess the effects of different soil managements on the microbial soil life, a field experiment was carried out in Etyek-Buda wine region. We evaluated four different soil managements of : OT: old vineyards + conventional tillage, OC: old vineyards + cover crop between the rows, FT: fresh plantation (2 years) + conventional tillage, FM: fresh plantation (1year) + conventional tillage + organic manure. Soil physical and chemical parameters (soil humidity, humus, labile C fractions, pH, CE) and biological parameters (DHA activity, glomalin) were measured at the level of the three soil depths (0-20cm), (20-40cm) and (40-60cm). Because of their relationship with soil functionality, the soil microbial activity have been proposed as useful indicator of both soil improvement and degradation in soil quality monitoring tasks.

This study aimed, first to compare the effects of different soil management and different cultivation techniques on vineyards, and how they influence the microbiological soil life, and second to highlight the importance of how sustainable soil management and other agro ecological practices can optimize the management and sustainability of vineyards.

From a soil-management perspective, the results showed positive implications and repercussions on all the studied soil parameters, as a result of the practice of cover cropping, no tillage and organic manure. In summary, we found that:

- First, long-term tillage practices affect soil characteristics like water content, aggregation, organic matter content, and enzymatic activity which, in turn, impact microbial diversity. Thus, it has become a well-known principle of conservation tillage that soil organic matter increases in the surface few centimeters of soil as tillage is decreased. This is especially true for no tilled soils, because plant residues accumulate at the surface and are not mechanically mixed into the plow layer.
- Second, cover crops are a relatively inexpensive method of augmenting carbon into the soil, resulting in benefits both to the soil and to the environment. High levels of soil biological activity are frequently listed as an indicator of soil vitality. In this study we found that planting cover crops stimulates biological activity in the soil, serving as an energy source for the ecological community in the root zone, known as the rhizosphere. Soil with vegetation supports higher microbial populations than tilled soil. Our results showed also that vegetation cover increased soil carbon in both stable and labile fractions.
- Third, organic manure had significantly increased the activity of biological activity, as compared to unamended soil. The results showed that the organic fertilizer caused a more pronounced increase in the majority of soil life indicators such as humus content, humus stability, dehydrogenase activity and glomalin concentration, which in turn, lead to greater impact on microbial community.

In conclusion, we can say that soil management in viticulture and sustainable strategies assume greater significance to improve the quality of modern viticulture. In this this review we aimed to highlight new agronomic techniques capable of enhancing the resilience of the system and contributing to conservation and ecosystem services provision, and we found that agrotechnical practices required in integrated and organic farming, namely

soil management with covering crop is an eco-friendly system which can maintain the soil health in terms of soil biological fertility and productivity.

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9. ATTACHEMENTS

1. Appendix 1: Declaration on authenticity and public assess of final mater's thesis
2. Appendix 2: Statement on consultation practices

Appendix 1.

DECLARATION

on authenticity and public assess of final mater's thesis

Student's name:	OTHMAN Salsabil
Student's Neptun ID:	JUJBO1
Title of the document:	Evaluation of soil microbiology and soil life in different viticulture cultivation techniques
Year of publication:	2023
Department:	Viticulture and Oenology

I declare that the submitted final essay/thesis/master's thesis/portfolio[1] is my own, original individual creation. Any parts taken from an another author's work are clearly marked, and listed in the table of contents.

If the statements above are not true, I acknowledge that the Final examination board excludes me from participation in the final exam, and I am only allowed to take final exam if I submit another final essay/thesis/master's thesis/portfolio.

Viewing and printing my submitted work in a PDF format is permitted. However, the modification of my submitted work shall not be permitted.

I acknowledge that the rules on Intellectual Property Management of Hungarian University of Agriculture and Life Sciences shall apply to my work as an intellectual property.

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[1]Please select the one that applies, and delete the other types.

Appendix 2.

STATEMENT ON CONSULTATION PRACTICES

As a supervisor of OTHMAN Salsabil (NEPTUN ID: JUJBO1), I here declare that the final master's thesis[2] has been reviewed by me, the student was informed about the requirements of literary sources management and its legal and ethical rules.

I recommend/don't recommend the final master's thesis to be defended in a final exam.

The document contains state secrets or professional secrets: yes no*[3]

Place and date: Budapest, 2023/05/01

Zsuzsanna Varga, PhD
Internal supervisor

2 Please select applicable and delete non-applicable.
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