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# Master Thesis

## **Impacts of under-trellis management on soil and vine vigour**

Submitted by

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## Statutory declaration

I hereby declare on oath that I have created the present master's thesis without any outside help and without the use of any sources and aids other than those specified, and that I have indicated as such passages taken from the sources, I used either verbatim or in substance. I have never submitted this work in its present or a similar form to any other examiner as a graded work. I am aware that breaches of conduct will be sanctioned ("use of unauthorized aids") and may lead to further legal action. In addition to the printed version, this thesis has been submitted as a digital file to the examiner in charge of verification of the above statement.

Krems, October 16<sup>th</sup>, 2021  
site, date

Nicole Mayer, BA BSc  
name, signature

## Expression of thanks

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*“Great things never came from comfort zones.” [Anonymous]*

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*“Live a Life you will remember.” [Avici]*

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*“There is nothing more amazing, than being yourself.” [Darren Criss]*

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## Abstract

Beneath the various suitable possibilities in soil management of vineyards, the under-trellis management strategy plays a major role in maintaining the vitality of the vineyard environment and the grape vine itself. In the present master's thesis the influence of five different under-trellis management systems on the soil vitality and the grape vine vigour was investigated. Therefore five management systems were investigated: seeded cover crops, herbicide, natural vegetation, rotary tillage and under vine weeder (UVW). For the elicitation of the influence on the soil, the parameters soil humidity and enzyme activity were measured. The number of grape clusters, the yield and the ripeness were investigated to express the influence of under-trellis management on the grape vine. Another aim of this study was to examine the influence of an additional treatment with a mulch layer. Therefore, on the three management systems "Herbicide", "Cover crops" and "Rotary tillage" a mulch layer was added. Soil moisture strongly depended on the precipitation rates. In a depth of 0-10 cm the mechanical tillage influenced soil moisture negatively. The usage of a rotary tiller and finger weeder led to the most loss of soil moisture. Furthermore, it is important to point out, that a difference in soil moisture between "Rotary tillage" and "Herbicide" treatment in a depth of 10-20 cm is evident. The herbicide treatment showed the highest soil moisture content, but exceedingly few enzyme activity rates. The highest enzyme activity rates of phosphatase and  $\beta$ -glucosidase were measured in the treatment "Greening". Furthermore, the additional treatments with a mulch layer increased the soil moisture and the enzyme activity, primarily at 0-10 cm depth.

The treatments "UVW" and "Herbicide" showed the least soluble solids. Berry and stock weight were not significantly influenced by the treatments.

An implementation of no-tilled under-trellis systems, excluding herbicide application, could be a viable alternative to boost soil quality and enhance vine vigour. Nevertheless, to get holistic information, the effects of under-trellis management systems require further long-term studies.

## Zusammenfassung

Neben den verschiedenen Möglichkeiten der Bodenbewirtschaftung der Fahrgasse im Weinberg spielt die Unterstockbodenbearbeitung eine wichtige Rolle für die Erhaltung der Vitalität des Weingartens und der Rebe. In der vorliegenden Masterarbeit wurde der Einfluss von fünf Systemen der Unterstockbodenbearbeitung auf die Bodenvitalität und die Wuchskraft der Reben untersucht. Es wurden die Bearbeitungsvarianten: Herbizid, eingesäte Begrünung, natürlicher Bewuchs, Stockräumer und Rollhacke untersucht. Zur Ermittlung des Einflusses der Unterstockbodenbearbeitung auf den Boden wurden die Bodenfeuchte und Enzymaktivität gemessen. Der Einfluss der Bearbeitungsvarianten im Unterstockbereich auf die Rebe wurde anhand der Traubenanzahl, dem Ertrag und verschiedener Reifeparameter untersucht. Ein weiteres Ziel dieser Studie war die Untersuchung der Auswirkungen einer Grasmulch im Unterstockbereich in den Varianten „Herbizid“, „Begrünung“ und „Stockräumer“ auf Bodenfeuchte und Enzymaktivität. Es konnte eine signifikante Abhängigkeit der Bodenfeuchte vom Niederschlag in den Standardvarianten ohne Mulch erhoben werden. In einer Tiefe von 0-10 cm wirkte sich die mechanische Bodenbearbeitung negativ auf die Bodenfeuchte aus. Der Einsatz eines Stockräumers verursachte den größten Verlust an Bodenfeuchte im Bereich 0-10 cm. Die Variante „Roll-Fingerhacke“ führte zum höchsten Verlust an Bodenfeuchte in einer Riefe von 10-20 cm. Die Behandlung des Unterstockbereiches mit Herbizid zeigte den höchsten Gehalt an Bodenfeuchte, aber die niedrigsten Enzymaktivitätsraten. Die höchste enzymatische Aktivität von Phosphatase und  $\beta$ -Glucosidase wurde in der Bodenbearbeitungsvariante "Begrünung" gemessen. Die Ausbringung einer Grasmulch erhöht die Bodenfeuchtigkeit und Enzymaktivität in einer Bodentiefe von 0-10 cm in allen Mulchvarianten. In der Variante „Stockräumer“ und „Herbizid“ wurden die geringsten Mengen an löslichen Feststoffen im Most gemessen. Das Beeren- und Stockgewicht wurden nicht signifikant von der Behandlungsvariante beeinflusst. Der Verzicht auf mechanische Bodenbearbeitung und Herbizideinsatz im Unterstockbereich könnte eine praktikable Alternative darstellen, um die Bodenqualität zu verbessern und die Vitalität der Reben zu steigern. Zum Erhalt ganzheitlicher Informationen sind weitere Langzeitstudien erforderlich.

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## Introduction

Recent studies exhibit that cover crops and natural vegetation can increase biomass and microbial activity of soil in vineyards (Pérez-Álvarez et al., 2015). Biological activity is an indicator for high soil quality. Under-trellis techniques varies microbial composition and soil quality (Hendgen et al., 2018). The impacts of under-trellis management strategies like tillage, cover crops, mulch and herbicides need to be considered.

Soils perform certain functions that are essential for the whole ecosystem (Amelung et al., 2018). Soil quality is a leading factor for vine vigour (Muscas et al., 2017). Soil quality is defined by the potential of soil, that can regulate water movement, sustain plant biomass and animal life, filter pollutants, maintain nutrient cycles and preserve soil functions. A healthy soil helps the vine to keep in balance (Amelung et al., 2018) therefor it is necessary to focus on parameters for soil and vine vigour.

A healthy soil provides the fundament for a sustainable and ecological production of grapes. Currently, a discernible trend towards sustainable, biodiversity-promoting and resource-conserving vineyard management is visible. Winegrowers are striving to protect nature and to find new ways for vineyard management. Customers' demand for sustainable produced wines, the increased occurrence of extreme weather situations and the possibility to cultivate vineyards in a resource-saving way have been motivations for this master`s thesis. The vitality of soils is well known for its direct influence on the vitality of vine. Therefore, changes in soil management strategies are superior prospects to safe vine vigour. The effects of different under-trellis management strategies on soil enzymes, soil moisture, biomass of soils, ripening parameters of vine and weed suppression have to be demonstrated. In case, that these effects can be assessed and used to support vitality of vine.

Thereby we followed the hypothesis that the soil moisture content and enzyme activity of the soil are strongly affected by the under-trellis treatment. Furthermore, we addressed specially the hypothesis that a mulch layer strongly correlates with the content of the soil moisture. Methods to gain information to answer the hypothesis of this master`s thesis are the evaluation of soil moisture, activity of enzymes, yield and ripeness.

## 1 Vigour of soil and grapevine

Vigour is the ability to thrive and survive under environmental conditions. In ecology, vigour also refers to the competitive ability of species (Schopfer & Brennicke, 2011).

In addition to the important factor of harvest quantity, the quality and ripeness of the grapes also play a crucial role. In order to achieve these parameters in the best possible way, a balanced growth of the vine is needed (Smart and Robinson, 1991; (Kliewer and Dokoozlian, 2005). Cultivation of grapevine has to be managed and planned in detail, as pruning, canopy management and fertilization have a great impact on grapevine vigour (Bauer et al., 2017).

Despite the influencing factors induced by the vineyard, the balanced growth is influenced by the grape varieties, the planned yields and desired wine styles. For winegrowers it is often easier to increase their harvest quantities than the available water and nutrient content of the soil. The reduction of plant growth and the limitation of soil resources can be seen as an holistic challenge (Hickey et al., 2016).

Soil management strategies and fertilization have a strong influence on soil structure, water and nutrient availability and soil degradation. The aim of each winegrower should be to keep a balance between tillage, cover crops, fertilization and irrigation (Bauer et al., 2017). Soil management strategies have a strong influence on soil structure, water and nutrient availability and soil degradation. The wrong management in time and intensity can negatively impact biodiversity, soil physical parameters, humus degradation and mineralization and could also lead to soil erosion and the contamination of the ground water and the decline of soil fertility affects soil vigour indirectly. Winegrowers have the ability to manipulate the competition for water and nutrients by inter-row and under-trellis management-strategies (Winter et al., 2018). Complementary, (Buchholz et al., 2017) are convinced that plants and soil quality influence soil biota in vineyards mainly. Therefore, it is necessary to have a look on the soil and vine parameters.

## 1.1 Parameters of vital vine

### 1.1.1 Growth rate and wood-mature

An inadequate water or nutrient status leads to a strong limitation of ripening and growth rate of the grape vine. Furthermore light interception and leaf function are disturbed by these stress factors (Kliewer and Dokoozlian, 2005). In case of the supply of plants with needed nutrients an increasing fertilization of a limiting nutrient or other factors can result in plant growth, but in turn an oversupply of nutrients or water causes stress in the plant and thus also stress symptoms (Schubert, 2006).

The canopy density and the vegetative growth can be influenced by the planting density but also by the under-trellis ground cover. It was particularly evident that the vegetative growth was mainly influenced around flowering. In comparison, the shoot length of the grape vine can be correlated with the mid-day water potential of the stem. Even a stem water potential lower than -0.9 MPa results in a reduced growth rate.

The highest reduction of vegetative growth can be registered in treatments with higher plant density and more intensive under-trellis cover crops (Coniberti, Ferrari, Disegna, Garcia Petillo, et al., 2018). From this, it is evident that treatments with cover crops have a higher impact on the plant growth than the plant density, whereas the pruning weight are determined by crop soil coverage and soil type (Delpuech and Metay, 2018). An extensive vegetative growth often results in canopy self-shading at the under-trellis area. Furthermore intensive growth causes an increased expenditure on plant protection and a decreased wine quality (Jackson et al., 1993). Looking at this aspect from a practical perspective an argument against extensive vegetative growth are higher costs for canopy management (Bauer et al., 2017). Smart and Robinson (1991) stated, that an optimum growth rate of vine is between 0.30-0.60 kg PW (pruning weight) per m.

Regarding the wood maturity of the grape vine extremely strong shoot growth delays the development of the wood maturity. The wooden part of a grape vine serves for transportation of water and nutrients, for storage of the nutrients as a reservoir and the strengthening of the shoots. Therefore, a well matured wood is needed to improve frost resistance of the grapevine and is further important for the formation of moderate shoots and roots in the following growing season. Bauer et al. (2017) suggests a pith-to-wood ratio of 1:3. Furthermore, irrigation and fertilisation in autumn have a negative influence on the maturation of the wood, which manifests itself as delayed wood maturity (Bauer et al., 2017).

### 1.1.2 Water potential

The hydrologic balance of a vine is determined by the absorption of water via roots and the transpiration rate of the leaves. Primarily, precipitation between April and October influences vine growth significantly.

Water occupies an essential role in all metabolic processes in the vine and is needed to maintain vital functions and target growth. In general, water transport from soil to plant and further to the atmosphere is managed by the water potential concept. Therefore, Energy is needed to transport the water from higher water potential levels to lower ones (Hoppmann et al., 2017).

Figure 1 shows the gradient of water potential from the soil, roots, stem, leaves and atmosphere at a high and low gradient. Vine can extract water from soil from usable field capacity of -0,032 MPa up to a water potential of -1.6 MPa (permanent wilting point of the soil). The increasing gradient is described as the driving force for the water transport. In order to transport water across the vine, the potential values from the root to the stem and shoot to the leaf, have to become smaller. There are meteorological, vine physiological, soil physical and viticultural influences on the water balance of the vine (Hoppmann et al., 2017).

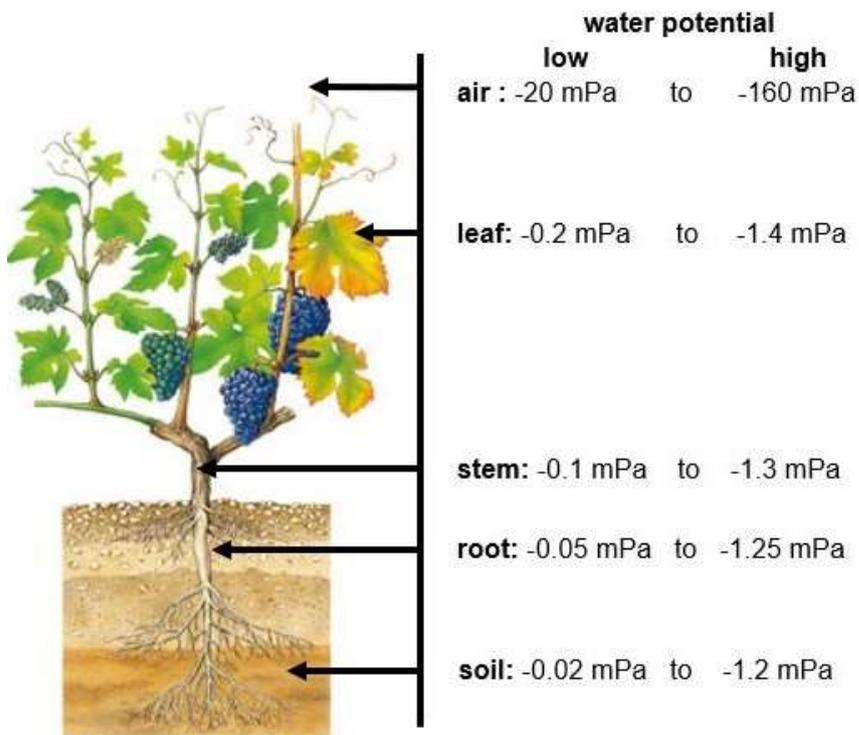


Figure 1: Water potential gradient of a vine, retrieved (weinkenner.de ,2021)

The water potential of stem is not influenced by soil treatments like tillage, but it decreases over the growing season (Steenwerth et al., 2016).

### 1.1.3 Yield

Modern agriculture systems focus on maximum yields and optimize wine production without a trace on negative impacts on environment. Winegrowers have to track a suitable yield to earn enough money and handle long-term ecosystem services (Winter et al., 2018). Therefore, vineyard management has to be adopted to current circumstances to adapt to local climate, face changing weather conditions and aspire a moderate wine production (Steenwerth and Guerra, 2012). Water and plant nutrients serve as building blocks of plant metabolism and therefore they influence the yield capacity of a vine (Schubert, 2006). Under-trellis management has an influence on several components of yield (Hickey et al., 2016). Compared to plant density, under-trellis management has a bigger effect on yield than the plant density. Berry size is positive correlated to vegetative growth and indirectly impacts yield (Coniberti, et al., 2018). Long-term productivity of vine and yield capacity are interlinked and have to be in a balanced ratio. Nevertheless it should be noted that high yields might pose quality problems (Bauer et al., 2017; Delpuech and Metay, 2018).

### 1.1.4 Photosynthetic capacity

The photosynthetic capacity of a vine primarily depends on solar radiation. Exposed leaves are heated up to more than 10 °C above the general air temperature. It is well known that grape-bearing vines and shoots have a higher photosynthetic capacity than vegetative growing ones. In comparison shaded or inner leaves (2<sup>nd</sup> leaf layer) have a photosynthetic capacity of 33 % (Bauer et al., 2017). Generally, grape vine leaves reach their maximum efficiency after 35 days of growing and lose it until the end of vegetation. The limiting factor, water deficiency can reduce photosynthetic performance by stomata closing (Hoppmann et al., 2017). A leaf-fruit ratio (Leaf Index) of 1-1.2 m<sup>2</sup> of leaf area for 1 kg of grapes can be seen as the optimum. The leaf-fruit-ratio should allow the grape vine to store sufficient reserve materials, which are needed for the wood maturity. Generally, leaf area varies depending on location, growth rate, training system, variety and foliage work (Bauer et al., 2017). Vine-shading has a negative impact on the colour intensity of berries (R. Smart and Robinson, 1991), but a pruning weight above 0.6 kg leads to a little increase in photosynthetic capacity (Coniberti, et al., 2018).

### **1.1.5 Natural Resistance**

In nature, the stimulation of the plant's own defence mechanisms leads to induced resistance. An equilibrium between vine, pest or pathogen and its antagonists must be created. This can be reached by an establishment of a balanced ecosystem with optimized plant cultivation, maintenance measurements and the targeted use of plant fortifier (Hofmann et al., 2014). The preservation of the ecosystem, plant care and plant healing can promote a holistic enhancement of the grape vine. With the right maintenance measurements, the environment of the vineyard can be preserved. (Hofmann et al., 2014). Furthermore the ecosystem of vine, like hedgerows and buffer stripes, has to be promoted and soil fertility preserved (Garcia et al., 2018). Plant care includes the promotion of plant growth and strengthening of the grape vines to avoid weaking processes. Overall, long-term organic fertilizer strategies and plant nutrition have to be encouraged (Mbuthia et al., 2015). Factors like soil revitalization and the increase of the anti-phytopathogenic potential of the soil are important (Hofmann et al., 2014). The following influencing factors should be mentioned too: training systems of vineyards, microclimate, locations, varieties, rootstocks and soil parameters (Garcia et al., 2018). The mentioned factors are part of the regulating and supporting systems, which have an impact on the maintenance of the vineyards environment. Microorganisms and their metabolites, plant inhibitors microflora with antagonistic effect on pathogens, pheromones and beneficial insects can be used for the protection against pests attacking the grape vine (Hofmann et al., 2014). It is important to know, that plant healing relies on plant protection and plant fortifier to prevent the overgrowth of a pest (Hofmann et al., 2014). Nevertheless plant protection measurements should be used as a preventive action (Winter et al., 2018). Generally, the basic principle of resistance is to promote secondary ingredients such as phytoncide protective substances (pre-infectious resistance factors) to regulate and control metabolic processes, defend against pests and promote plant recovery (Hofmann et al., 2014).

## **1.2 Parameters of vital soils in vineyards**

### **1.2.1 Soil moisture and water regime**

The content of soil moisture plays a crucial role, because insufficient and excessive water supply of soil can cause stress on vine. Another negative result of stagnant water is the impairment of plant growth and reduced root respiration. In turn it leads to an inhibited nutrient uptake and malnutrition. In comparison, insufficient water supply

causes drought stress, which negatively affects the elongation growth of the cells and promotes an increased production of abscisic acid. The synthesis of abscisic acid leads to stomatal closure, inhibits meiosis, and causes low fertility (Schubert, 2006). A particularly critical stage for drought stress is flowering. Volumetric soil water content is strongly affected by rainfalls. Especially rainfalls in winter refill the water reservoirs of soils (Hoppmann et al., 2017). The influence of the rainfalls lead to differences between the treatments especially at lower soil depths and early in the vegetation period (Steenwerth et al., 2016).

It is known that a grape vine forms its most roots in a depth of 30 to 60 cm, which allows the most water absorption in this area (Smart and Robinson, 1991). No-till treatments have a lower volumetric soil water content in comparison to tilled treatments at all depths (Steenwerth et al., 2016). Due to this fact, we can estimate, that the volumetric soil water content of no-till treatments decreases caused by a delayed reduction of soil moisture. A layer of chopped or flattened cover crops can preserve soil moisture. The water up take of cover crops and vine are at different depths under standard conditions. In hint, vine compensates water deficiency by water uptake from upper layers (Steenwerth et al., 2016).

### **1.2.2 Humus content and biomass**

Organic matter is composed of approximately 90 % humus and 10 % biomass. The organic matter also has an indirect effect on pore size and the water balance due to its aggregating effect. In sandy soils, the humus content determines the field capacity positively (Amelung et al., 2018). In sandy soils, an additional yield up to 10 % (in comparison to mineral fertilization) can be reached by using organic matter (Schubert, 2006).

Humus consists of dead organic substance and has a fundamental effect on water-holding capacity of soils. It can store three to five times more water than its own weight. This ability of absorption and storage of water can be used for revitalisation of the soil (Amelung et al., 2018; Heisteringer and Grand, 2014). The humus content of soil supports the supply of vine. Plant nutrients can be mineralized from the humus particles and bound on humus particles of soils. This mechanism shows the importance of the humus content for the nutrient supply of the grape vine. The humus concentration of a soil is not constant. It is influenced by decomposition and build-up soil processes. Three essential variables of humus balance are the initial soil material, the location and the cultivation (Schubert, 2006).

The biomass and vital organic matter comprises soil fauna, flora and plant roots (Schubert, 2006). A higher plant biomass does not only lead to an increase in species and density of earthworms. Moreover, soil biota is modified by plant biomass and soil quality. From this point of view, the soil quality is important for a diverse soil biodiversity. Earthworms and springtails are indicators for ecosystem service provision in vineyard soils. Fundamentally, the intensity of tillage has a major influence on soil biota (Briones and Schmidt, 2017), but less influence on plants or soil quality. Habitats surrounding the vineyard have an influence on biodiversity of soils, but not on the occurrence of earthworms (Buchholz et al., 2017).

### **1.2.3 Plant available nutrients**

For the maintenance of the vital functions, the grape vine absorbs its essential nutrients from the soil. Therefore, the presence of water and an optimum nutrient balance of soil guide the uptake of nutrients (Steenwerth et al., 2016). Vine absorbs nutrients from soil to maintain its vital functions (Schubert, 2006). The available concentration of nutrients in soils affects the supply of nutrients to the plant. Nutrients can be bound to soil particles or freely available in soil solution (Amelung et al., 2018). The availability of macronutrients Nitrogen (N) , Phosphor (P), Potassium (K) and Magnesium (Mg) for grapevine is highly interesting (Pérez-Álvarez et al., 2015). Nitrogen is essential for the vegetative growth and yield, therefore the mineralization of organic nitrogen to nitrate has to be triggered to redeliver low levels of nitrogen of soils (Pérez-Álvarez et al., 2013).

Another macronutrient of high importance is Potassium. It is immobile in soils and dissolved in soil solution as  $K^+$ -Ion to be taken up by the root tips and it regulates the sap flow and the grape acidity (Bauer et al., 2017; Pérez-Álvarez et al., 2015). E.g. high levels of Potassium decrease the free tartaric acid of the grapes and rises the pH of the grape juice (Mbuthia et al., 2015). Furthermore, the macronutrient Magnesium is important for the photosynthesis, blocking of chlorophyll and metabolism in general. Magnesium and Potassium control the water balance of the vine. Nevertheless, it has to be pointed out, that Magnesium is very mobile in the soil and a high content of Magnesium in the soil inhibits the uptake of Potassium. Phosphorus supports the metabolism of carbohydrates. It is a component of protein substances and plays a major role in the energy balance of the grape vine. Soluble Phosphate is actively absorbed by vine roots but Phosphor is poorly mobile in soils (Bauer et al., 2017).

#### **1.2.4 Soil texture and structure**

Soil texture is a basic parameter for development, productivity buffering and filter function of soils. It is defined as the particle size distribution of inorganic material. The soil particle sizes are itemized in sand (2.000-63  $\mu\text{m}$ ), silt (63-2  $\mu\text{m}$ ) and clay (<2  $\mu\text{m}$ ). The sand fraction contains sand and coarse pores. The clay fraction features clay with fine pores, less available water, and poor air capacity of soil. The silt fraction is intermediate clay and sand fraction with the highest water capacity. The shape of the soil particles can vary from angular or well-rounded after the impact of wind and water transportation. The porosity is strongly influenced by particle shape, size, mixture, and compaction. A compaction of soil could occur in case of mechanical disintegration like tillage with heavy machines. Naturally, a mixture of particle sizes appears in soils. Genetical formation and physico-mineralogical characteristics of parent material determine the final soil texture. The soil texture influences water potential, plasticity, structure stability, cation exchange capacity, ecology, and productivity. Water, air and solid volume relate to soil texture (Amelung et al., 2018).

Soil texture cannot explain spatial division of particles and pores on its own. Soil structure defines the characteristics of soil parameters. It is a dynamic and sensitive system to changes (Amelung et al., 2018). Therefore, winegrowers should focus on treatments, which promote soil conservation (Steenwerth et al., 2016). Weathering processes form soil texture and further aggregation. Texture, parent material, biomass, water, gas, microbes, stability are all soil structure influencing parameters. The soil formation factors and the combination of organic-inorganic components play a crucial role for the development of the soil structure. Mainly, weathering and decomposition processes are responsible for the formation of humus and the mineral body of a soil. Furthermore aggregation and segregation structure soils and its functionality (Amelung et al., 2018). Indicators of soil quality are the content of soil carbon (C), soil nitrogen (N), microbial biomass and community structure of biota. Other important indicators for soil quality are organic matter levels, greenhouse gas emissions and soil leaching of nitrate (Drijber et al., 2000). These soil quality factors can be defined by their contribution to crop productivity, nutrient cycle, and quality of environment. Exemplarily, humus supports fertility, organic matter storage and nutrients. Besides, pH of soils dictates nutrient availability. Furthermore the extractability of nutrients like N, P and K, bulk density or electrical conductivity are characteristic for soil quality (Arshad and Martin, 2002). Besides, the soil management assessment framework

(SMAF) can evaluate soil quality. SMAF is a ranking tool for sustainable soil quality by a three-step schema. Indicators must be selected, interpreted and integrated. Indicators like plant productivity, water and air quality or human and habitat health are considered for this framework (Andrews et al., 2004).

### **1.2.5 Microbial activity**

The entity of all organisms in soils is composed of individual populations of different species. The composition of the microorganisms is determined by the ecological living and habitat conditions such as climate, soil type or soil depth. Nutrients are heterogeneously distributed in soils and can be accessed differently by soil organisms. Soil bacteria composition depends on the transport of nutrients, because they are immobile whereas soil fungi can grow by hyphae to reach their food sources. Soil microhabitats with high availability of nutrients and the present of energy sources are soil areas next to plant roots, earthworm burrows and closely areas to soil litter (Amelung et al., 2018). The microbiome of soil effects biomass, nutrients, and buffer capacity of the soils. As example, bacterial species richness is significantly reduced under integrated management compared to organic managed soils. Organic vineyards exhibit higher fungal community composition than integrated vineyards (Hendgen et al., 2018). Balanced vigour should be aspired by winegrowers. Nowadays different soil management techniques of under-trellis area are at the disposal. Subsequently, opportunities of under-trellis cultivation like cover crops, herbicide, mulch, and tillage are conceived.

## **2 Soil management techniques of under-trellis area**

In many vineyards, the under-trellis area encompasses approximately 1/3 of the total vineyard area. Therefore, the under-trellis management plays a determinant role of vineyard management. Standard practices for maintaining the under-trellis area are tillage and the use of cover crops, herbicides or mulch (Hofmann et al., 2014). The appropriate actions can vary, depending on several factors. Soil management systems of a vineyard should be adapted to the age of the vines, local conditions, soil type and climatic conditions (Ripoche et al., 2010). Primarily, under-trellis management serves to guide the vegetation, protect the soil, control the water and nutrient balance, and improve the soil biodiversity (Ripoche et al., 2010; Steenwerth and Belina, 2008). In general, the effects of different under-trellis management strategies are well known among winegrowers. Following, the four most widely used under-trellis maintenance measures will be explained.

### **2.1 Under-trellis cover crops**

Under-trellis cover crops provide soil cover which helps to reduce erosion and promotes biodiversity of vineyards. Furthermore, they can improve root performance of the vines, and reduce nutrient leaching. Low-growing cover crops, seeded or alternatively naturally growing plants, should be promoted to create a vegetation cover (Hofmann et al., 2014).

### **2.2 Herbicides**

Herbicides are used to control weeds and can be selective or non-selective. The active ingredient can interfere with different targets in the plants' metabolism and causes plant death within a short period of time. Low dose rates of herbicides are sprayed with panels to prevent drift. There are contact herbicides and systemic herbicides available (Bauer et al., 2017). As an example, the active substance glyphosate affects all green plants by inhibiting the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPs), an enzyme of the shikimate pathway. The shikimate pathway is a metabolic pathway in plants for the synthesis of the aromatic amino acids tryptophan, tyrosine and phenylalanine (Siehl, 1997). The lockdown of shikimate pathway decreases flavonoid and lignin concentrations in plants (Olesen and Cedergreen, 2010). In a one-year study comparing mechanical to herbicide treatment. Donnini et al. (2016) could not

determine significant differences in fresh berry weight, total soluble solids, pH of berry juice and titratable acidity.

Herbicides decrease grapevine mycorrhization and soil total colony-forming units (Zaller et al., 2018). The reproduction of earthworms' biomass, density and surface activity are not affected. Content of Magnesium in leaves and Nitrogen in grape juice differs between mechanical and chemical treatments (Zaller et al., 2018). High efficiency combined with low expenses and easy handling are the main advantages of herbicides. Development of herbicide resistances and toxicity to vine and operator are the main potential disadvantages. A control of annual weeds by herbicides may lead to an increased incidence of perennial weeds (Elmore et al., 1997). In international viticulture, glyphosate is currently the most widely used herbicide for weed management (Siehl, 1997).

### **2.3 Tillage**

The under-trellis area is mechanically tilled to repack the soil surface and reduce the usage of herbicides (Merwin et al., 1994). The aim to reduce the application of herbicides leads to an increased use of mechanical tillage equipment in the last years (Steenwerth and Guerra, 2012). The technical possibilities are diverse and have to be site-adapted (Hofmann et al., 2014). The decision for an appropriate technique has to be based on the management system, soil type, winery structure, soil condition and slope. The priority selection criteria are working speed, quality of work, injuries to the vines, time and cost expenditure, repair and maintenance effort, and the sustainability of the measures in weed control (Walg, 2010). The cultivation of the under-trellis area by under-vine weeder (UVW) is popular (Hofmann et al., 2014). The UVW can have a scraper or a flat coulter. Swinging in and holding work position of the coulters in the row of vines is done under pressure. A feeler is used to move around the vines. The feeler initiates the coulter to swing out of the row. The best-known variant is the flat coulter. A flat coulter undercuts the vegetation and cuts the roots of weeds. Advantageously, tillage by flat coulter does not shift soil. A negative aspect is that weeds can regrow in case of rainfalls directly after tillage. Working depth of the flat coulter is between 5-8 cm (Walg, 2010).

## **2.4 Mulch**

Mulch is an organic material, that can be placed on soil surface to suppress weeds and maintain the soil moisture. The minimum thickness of a mulch layer should be 10 cm to block light and successfully prevent the emerge of undesirable seed weeds (Steenwerth and Guerra, 2012).

### **2.4.1 Weed management**

Under-trellis cover with organic materials causes a lack of light for germination of seeds. Generally, mulch layers provide facilitated development conditions for root weeds, like couch grass and bindweed (Bauer et al., 2017). The usage of mulch as a weed cover depends on application date and the last establishment. Weed-free stripes are not recommendable in established vineyards with sufficient resources, because of the possible high erosion (Bavougian and Read, 2018). Weed control under wider mulch layers is more effective, but the effectiveness of weed control declines over time caused by the organic degradation of the material (Chan et al., 2010).

### **2.4.2 Soil temperature**

The soil temperature is influenced by a mulch layer. The respiration of microbes, rate of organic matter decomposition and nutrients availability are favoured by soil temperature. Furthermore, the soil temperature influences the growth of shoots, the cytokinin content in vine roots and the yield. In comparison, a low soil temperature benefits the formation of leaf mass, fruit sets and rise of berry cluster weight (Amelung et al., 2018). In cool climates a premature bud-break, caused by higher soil temperature, has to be considered and therefore also the rise of risk of frost damage (Sandler et al., 2009). An important influencing factor of soil temperature is the date. In addition to the season, a grass mulch layer raises soil temperature to higher degrees as compared to exposed soils. An increased activity of soil microbes is a possible reason for higher soil temperature under a grass mulch layer. The heat conduction between straw mulch and soil surface supports the capability of mulch to moderate soil temperature. In comparison to mulch, cover crops the soil temperature, because of higher transpiration rates of the living plants. Nevertheless, further studies show an insufficient difference in temperature between mulch, bare soil and greened treatments (2 °C, approximately) (Bavougian and Read, 2018). In addition, a study in California, conducted by Steenwerth and Belina revealed, that grain groundcover has no influence on soil temperature compared to bare soils. Mulch layers reduced the daily soil

temperature amplitude already at a depth of 10 cm. These contradictions show the need for further research (Steenwerth and Belina, 2008).

### **2.4.3 Soil and vine water content**

Different plant species have different evaporation rates and therefore also different consequences on the soil moisture content. Soil moisture under a mulch layer is higher compared to herbicide, cover crops and bare soils (Bavougian and Read, 2018). Basically, soil moisture is essential for fauna and flora of the soils. As an example, increased activity of microbes has been measured in soils of mulched green-cover inter-rows, caused by proper soil water content (Jacometti, 2007). Soils under straw mulch possess the greatest water ability and intermediate water potential at herbicides treatment (Merwin et al., 1994). Mulch layers save water, but there is no recognizable difference between slim (30 cm) or wide (60 cm) treatments. A high water content can be justified by a combination of reduced evaporation loss, greater soil water conservation and diminished weed growth (Chan et al., 2010).

### **2.4.4 Retro-reflective effect**

The quantity and type of reflected radiation is affected by soil quality, vegetation and mulch at under-trellis area (Meinhold et al., 2010) and by the canopy density. The canopy can transmit a part of reflected light, nevertheless, attention should be paid to the differences in transmittance. These differences can occur, depending on the date of measurement before canopy closure. Mulch layers have to be preserved and free of debris (Sandler et al., 2009). Especially, the photosynthetically active radiation (PAR) reflectance should be remembered. The PAR reflexions of bare soil are lower compared to different types of mulch. When vineyard floors reflect radiation light, this leads to PAR interception and might cause changes in fruit composition (Smart et al., 1988). Contributed grass mulch followed by mowing of inter-rows can alter the surface reflection (Bavougian and Read, 2018).

### **2.4.5 Soil fauna and flora**

A mulch layer has a crucial impact on the composition and amount of soil fauna and flora. The topsoil organic matter mostly raised (+0.9 g/kg) under straw mulch within four years (Addison et al., 2013). In contrast, the organic matter decreased under tillage treatments and pre-emergent herbicides. Long-term conservation of soil structure should be the aspirational target of every winegrower (Merwin et al., 1994). A mulch layer can lead to an increased occurrence of vole (Bauer et al., 2017) and has

an influence on the soil arthropods. Results show that mulch layers foster a variety of arthropods. In comparison, treatments without a mulch layer can exhibit higher amounts of pest insects like fruit flies and grasshoppers (Addison et al., 2013). Surprisingly, the frequency of springtails and ants were higher in non-mulched treatments. This result is based on the fact, that springtails are prey for many predators and more springtail predators are found in the mulch variant. Ants and excrement beetles can be considered as indicators of disturbance, minor soil quality and intensity of land use. A richness of insects under mulch results in higher numbers of omnivores (Addison et al., 2013).

Most of the vineyards are frequently and intensively cultivated by pesticide treatments and tillage (Buchholz et al., 2017). Therefore mulches are an effective alternative of vineyard management in comparison to mechanical under-trellis management. It has to be mentioned, that soils under a grass mulch layer have a higher volumetric water potential and grass mulch only have to be applied two times to control weed. Furthermore, winegrowers should use a local inexpensive source to produce grass mulch, because transportation costs and application are tremendously high and mostly economically justifiable (Bavougian and Read, 2018).

### **3 Influence of under-trellis strategies by cover crops and tillage in vineyards**

Ecologists are concerned about the loss of biodiversity and the decline of ecological degradation. Simultaneously, winegrowers try to increase yields, minimize costs and improve the conditions for their production (Tilman et al., 2002). The establishment of permanent cover-crops or permitting resident vegetation reduces the requirements of synthetic chemicals and tender habitat for arthropods and other soil organisms (Bavougian and Read, 2018).

#### **3.1 Soil moisture and soil quality**

Cover crops or mulch layers support water infiltration and can enhance soil structure (Hartwig et al., 2002). Especially, plant communities have an influence on the soil moisture (Lange et al., 2014), whereas, under-trellis ground cover like herbs and grass have no significant influence on the water potential of soils. A difference between grass coverage and herbicide treatment has been determined in special depths and seasonal phases. At 60 cm depth, the herbicide treatments had 24 % more soil moisture than the treatment with grass coverage. Generally, soil moisture was lower at 10 to 40 cm than in 60 cm. These differences have been more evident in the herbicide treatments than in the other ones. Vine roots take water from shallower layers (Hickey et al., 2016). Grass cover crops with Creeping Red Fescue provides a competition in early vegetation season resulting in the reduction of the vine growth. Creeping Red Fescue and similar grass cease their growth at periods of drought stress (Giese et al., 2015). Influences of under-trellis ground cover depends on seasonal progress. Comparing treatments, variation of soil moisture in mid-summer season is lower and herbicide treatments have a worse volumetric soil water content in late summer (Hickey et al., 2016). Special cover crops for cool season operate as a mulch layer reducing rainfall infiltration and enhancing evaporation rates. Therefore, soil moisture increases compared to mechanical tillage (Celette et al., 2008).

Deceased or mowed cover crops reduce negative effects on volumetric soil water content caused by cover crop regrowth, especially in no-till treatments (Steenwerth et al., 2016). Findings suggest that vineyard floor vegetation influences vine, but not in competition of water (Bavougian and Read, 2018). Volumetric soil water content declines over growing season, but it does not depend on tillage or cover crops. Soil

treatments like tillage and cover crops effect volumetric water content of soils, but do not affect stem water potential. Water uptake by cover crops and vine take place at different soil layers. Vine compensates water deficiencies caused by water competitive situations in shallow layers by root growth. Tilled treatments have higher water potential in all soil depths than no-till treatments (Steenwerth et al., 2016). Effects of soil management strategies like cover crops or reduced tillage on soil quality need time (Al-Kaisi et al., 2005).

### **3.2 Nutrients, vine growth and yield**

Nutrient dynamics are operated by mulch layers and cover crops (Hartwig et al., 2002). Giese et al. mentioned that, cover crops sequent mineral nutrients, mainly nitrogen (Celette et al., 2009; Giese et al., 2014), whereas Coniberti et al. could not find any significant effect on tissue nutrient status by ground cover (Coniberti, et al., 2018). Cover crops can stimulate excessive vegetative growth of vines (Giese et al., 2014), but in glasshouse trials the ground cover treatments haven't been affected directly (Vukicevich et al., 2018).

Over the time, the impact of under-trellis cover crops on vine growth has been reduced, caused by the ability of vine roots to grow into deeper soil layers (Hickey et al., 2016) to avoid competition with the cover crops (Klodd et al., 2016).

Plant tissue analysis show, that quantity of most nutrients has not been influenced by under-trellis ground cover whereas in comparison, the treatments affected the nitrogen (N), potassium (K), phosphor (P) and magnesium (Mg) content (Hickey et al., 2016). The effects of nutrient deficiency sometimes occur several years after establishment of cover crops (Pérez-Álvarez et al., 2015). Furthermore, the important factors in soil chemistry like pH and NO<sub>3</sub>-N are marginal decreased within ground covered soils in contrast a rise of the NO<sub>3</sub>-N content by N-mineralization is caused by water input. This fact can be defined, cause the water-input increases the mineralization of labile soil organic matter in coarse textured soils (Vukicevich et al., 2018). Nitrogen reserves may have an influence on reduced yield (Hickey et al., 2016). Under-trellis ground cover treatments with N-fixing legumes and sufficient water supply enhance the availability of soil resources and reduce microbial diversification (Vukicevich et al., 2018). In contrast, a permanent ground cover enhances organic carbon, aggregation of particles and biological activity of soils in vineyards (Peregrina et al., 2014). Furthermore, these soil conditions affect growth and nutrient uptake (Pérez-Álvarez et al., 2015). It is

important to point out, that it is difficult to distinguish the effects of nitrogen and soil moisture on vine growth (Hickey et al., 2016). Losses of NO<sub>3</sub>-N during bloom reduces yield and vine vigour (Pérez-Álvarez et al., 2013). As an example at bloom and veraison, the petioles and leaf blades of grape vines in grass coverage treatments possess lower N-content in the first year after establishing a grass cover (Hickey et al., 2016).

Mechanical tillage, clover and barley cover crops neither significantly influence cluster number per vine nor yield (Pérez-Álvarez et al., 2015).

### **3.3 Biodiversity, microbial density and composition**

Diverse groundcover vegetation influences the content and composition of soil microbes (Whitelaw-Weckert et al., 2007) whereas, a high plant variety increases the antagonistic microbial communities and may decrease the success of pathogens (Latz et al., 2012). Investigations of the microbial mixture of grape must and wine shows regional and vineyard related differences, therefore the importance of vineyard management strategies can be assessed (Bokulich et al., 2016). The biological diversity of microbes in vineyards plays an important role for the development of the regional wine typicity. This regional difference in the composition of the biological diversity is called “microbial terroir”. A so called “microbial terroir” can be defined as a collection of bacteria and fungi of a special area, that contributes to a typical wine style (Belda et al., 2017). Furthermore, ground cover like a mulch layer is important, because it supports the appearance and settling of beneficial insects to enhance the power of biological control (Simon et al., 2009).

Regarding to soil-borne organisms, springtail diversity is not influenced by tilled treatments, but the springtail density, mainly the greater species, are reduced in permanently green covered soils. A high richness of earthworms originates from soil management, plant biomass and its interaction with soil quality. Generally, vineyards with high soil quality represent high levels of soil biota. The content of species in soils plant biomass increases simultaneously. This effect is especially recognizable in tilling treatments. Thereby the litter decomposition dependencies strongly depend on tillage and ground cover management strategies, biomass, and soil quality. In contrast, microorganisms in soils are less affected by tillage and landscape than by the plant biomass and soil quality (Buchholz et al., 2017).

Soil-organisms and plant communities, caused by natural coverage of the soil in vineyards, play a major role in biodiversity. The different groundcover communities like seeded grass and naturally occurring cover crops do not differ in root weight, but all groundcover treatments tend to smaller root systems and influence root necrosis compared to bare soil (Vukicevich et al., 2018).

Concerning biodiversity, soil-borne fungi are considered as essential. Hendgen et al. stated, that under-trellis cover crops enhance the fungal richness of soils (Hendgen et al., 2018). Regarding this statement, the effects on fungal composition could be disguised by climate, soil type, cultivation system, vineyard history or inter-row vineyard management. Results indicate that vineyard microbes are manipulable by under-trellis practices (Chou et al., 2018).

In a three-year average, the coverage rate of soil vegetation under natural vegetation is 70 % greater than mechanical cultivated and herbicidal treatment. Differences in biodiversity of soil between the treatments “natural vegetation” compared to “herbicides” and “mechanical tillage” increases by progressing implementation. Changes in soil microbial composition, do not extend to fungal communities on grapes. Only year-to-year differences in microbial composition of grapes are significant (Chou et al., 2018).

The usage of reduced tillage and cover crops are linked to a higher microbial activity and therefore improved soil quality. Other indicators for soil quality are the carbon, nitrogen, and phosphate metabolizing enzymes ( $\beta$ -glucosidase,  $\beta$ -glucosaminidase and phosphodiesterase). In no-till treatments, these metabolizing enzymes show higher appearance rates than in treatments with tillage.

These enzymes play an important role in the maintenance of soil-nutrient-balance, e.g., a low activity of  $\beta$ -glucosaminidase can lead to a rise of nitrogen rates and a decrease of the mycorrhizae fungi content of the soil. Vetch as cover crops minimize the fungi mycorrhizae biomarkers and result in higher  $\beta$ -glucosaminidase and basal microbial respiration rates. Higher rates of total organic carbon and  $\beta$ -glucosidase in no-tilled treatments are indicators for soil quality (Mbuthia et al., 2015).

Management systems like cover crops, canopy height and phyto-sanitation can modify soil conditions and the transport of microbes from the soil to the grapes. This translocation could capture a critical role in fruit shaping and wine composition (Chou et al., 2018). The potential yield of under-trellis cover crops treatment has been reduced compared to herbicide treatments. Furthermore, grass coverage treatment

presents higher levels of soluble solids (Brix°) compared to herbicide treated under-trellis areas. Tartaric acid content of berries do not significantly depend on treatment (Hickey et al., 2016). Furthermore the total berry skin anthocyanins and phenolics were not influenced by the under-trellis ground cover (Hickey et al., 2016). Herbicide treatments have to be harvested earlier, because botrytis bunch reached 10 % decay of berries at first (Coniberti, et al., 2018).

The existence of cover crops, despite of type, leads to a variation of the weed community mixture. A low intensity of tillage influences weed community composition already after three years. In contrast, cover crops reduce the variety and richness of weed community compared to natural vegetations and tillage. Basically, it can be seen, that plant biomass differs by year and treatment. The total nitrogen content of biomass depends on the cover crops. Legume accentuated variants show a higher total nitrogen content of biomass. Likewise, the C:N ratio in aboveground weed biomass is influenced by tillage, caused by the return of fresh biomass to soil. Steenwerth, et al., 2016 proved that mechanical tillage makes nitrogen more available for plant uptake. Inorganic nitrogen amounts and potential mineralized nitrogen do not depend on tillage or cover crops (Steenwerth et al., 2016).

## 4 Material and methods

### 4.1 Experimental vineyard

The experiment is conducted in a vineyard in Lower Austria, Langenlois, terroir “Rosenhügel”, which was planted in 2008 with Pinot noir clones 18 Gm and 1-84 Gm and crafted on rootstock Kober 5BB (Figure 2) vineyard at the beginning of the vegetation period.



Figure 2: Study site at Langenlois, Nicole Mayer (2020)

The trial is established on an experimental vineyard of BOKU, Institute of Viticulture and Pomology, which is managed by the School of Viticulture and Pomology, Krems. Treatments has been established in 2018 and the experimental design is shown on Figure 4.

#### 4.1.1 Climatic conditions

Climate data is obtained from a weather station (company Adcon) at Langenlois-Steinhaus, next to the experimental vineyard. The climate data, given in Table 1, shows the sum of precipitation during vegetation period from February to September 2020 and the average temperature per month in °C. The total precipitation rate during the vegetation period from February to September 2020 is 378 mm.

Table 1: Monthly precipitation rate and average temperature from February to September in 2020 at Langenlois, Lower Austria next to the experimental vineyard

vegetation period (February to September 2020)	Sum of precipitation in mm / month	Average temperature in °C / month
February	0.0	6.2
March	17.4	6.3
April	4.6	11.6
May	37.4	13.6
June	86.4	18.2
July	71.4	19.8
August	87.6	20.8
September	83.0	15.8
<b>Sum of precipitation</b>	<b>387.8</b>	

#### 4.1.2 Soil conditions

The soil type at experimental vineyard is a chernozem with high lime content. A topsoil layer of more than 70 cm loess, a high usable field capacity of 220 to 300 mm and a moderate humus content facilitates the growing of vine. Negatively, soil parameters at study site have a moderate dry water potential, that may lead to water deficit (Table 2).

Table 2: Soil parameters of experimental vineyard (Bundesforschungs- und Ausbildungszentrum für Wald, 2021)

<b>parent material</b>	leoss
<b>soil type</b>	chernozem
<b>soil texture</b>	loamy silt
<b>thoroughgoing</b>	deep (more than 70 cm topsoil)
<b>water potential</b>	moderate dry
<b>water permeability</b>	moderate
<b>humus content</b>	moderate
<b>lime content</b>	strongly calcereous
<b>soil reaction</b>	alkaline
<b>usable field capacity</b>	high (220-300 mm)
<b>nitrate retention capacity</b>	high (340 to <=420 mm)

### 4.1.3 Experimental setup

The vineyard is designed as an experimental field with a randomized split plot system with two Pinot Noir clones, shown on Figure 2. Five treatments in four repetitions: Greening with mowing, rotary tillage, under vine weeder (UVW), herbicide application and greening without mowing as control treatment has been established in 2018. Additionally, three grass mulch treatments have been applied in 2020 on plots of treatment I “Greening”, III “UVW” and IV “Herbicide” (Figure 3).

<b>I Greening</b>
<b>II Rotary tillage</b>
<b>III Uvw (Under vine weeder)</b>
<b>IV Herbicide</b>
<b>V Control</b>
<b>Grass mulch</b>

Figure 3: Legend of experimental setup in field of under-trellis treatments

Figure 4 shows the test set up at experimental vineyard. Block A to E comprises every treatment plot (I to V) once. In order to obtain statistically usable data, the treatments and experimental design are set up in rows, each repetition is a row of its own. The sample area comprises 20 rows, every row is cultivated by one variant of under-trellis treatment (I to V). E.g., in the first row (R1), the entire row is cultivated by the under-trellis cover crop treatment Greening with mowing. The samples have been taken from red bordered blocks. One row of treatment includes 6x5 grapevines.

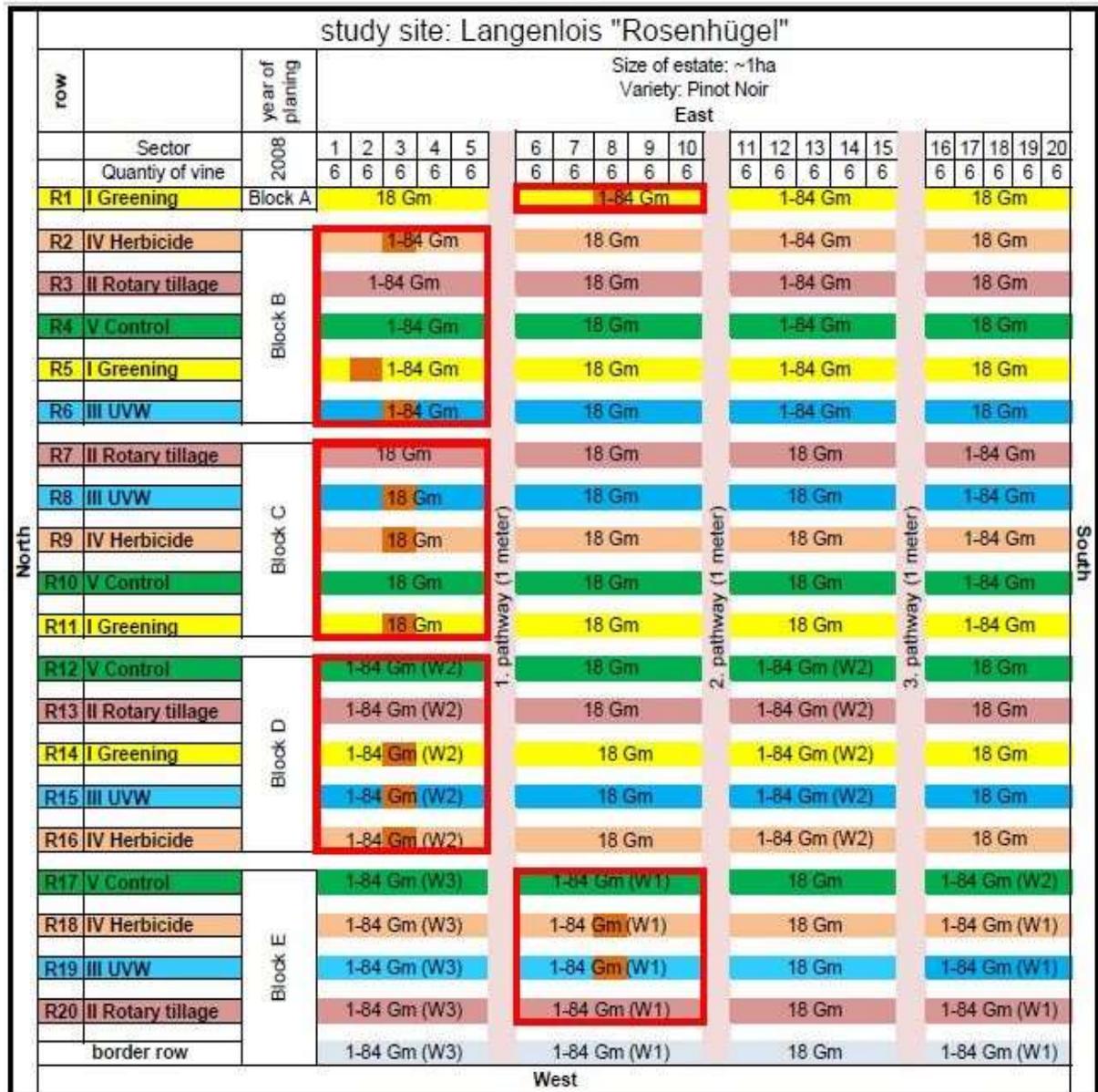


Figure 4: Plan of experimental set up (Block A to E), arrangements of repetitions, vines per variant and repeat on separate clones

#### 4.1.4 Cultivation and under-trellis management techniques

The vineyard “Rosenhügel” next to Langenlois is cultivated according to integrated production criteria. A spontaneous natural vegetation has been implemented in inter-rows und was mulched twice in 2020. Figure 5 shows the natural vegetation of inter-rows at study site.

Vineyard cultivation techniques, plant protection, fertilisation and inter-rows management is conducted uniformly across the entire study area.



Figure 5: Natural vegetation of inter-rows at study site, Mayer (2020)

The under-vine weeder (UVW) to implement treatment III “UVW” is shown on Figure 6. It is conducted with a stick scraper and clearing flat coulter fixed in the intermediate axle area. Under-trellis area is tilled by UVW on April 22<sup>nd</sup>, July 29<sup>th</sup>, and August 25<sup>th</sup>. The combination of rotary tiller and finger weeder for intermediate axle area to manage treatment II “Rotary tillage” is constituted on Figure 7. Under-trellis area is tilled by rotary tiller on April 22<sup>nd</sup>, July 30<sup>th</sup>, and August 26<sup>th</sup>.



Figure 6: Under-vine weeder (UVW) with flat coulter and rotary harrow, Mayer (2020)



Figure 7: Combination of rotary tiller and finger weeder, Mayer (2020)

Treatment I “Greening” has been established in 2018 and has to be mown in case of requirement twice in 2020 (June and August). Under-trellis area of treatment IV “Herbicide” is possessed on April 21<sup>st</sup> and August 10<sup>th</sup> by a systemic-acting herbicide “Roundup”.

Additionally, to the established treatments, a grass mulch has been applied three times throughout the growing season on the May 9<sup>th</sup>, June 17<sup>th</sup>, and August 28<sup>th</sup>. The implemented mulch layer is 5 cm thick and 50 cm wide, implemented on the under-trellis area.

## 4.2 Measurements and analyses

In order to investigate the effects of treatments on soil and vine vitality, the following measurements and analyses are conducted: activity of enzymes, soil moisture, photosynthetic capacity, water potential, yield and parameters of ripeness.

### 4.2.1 Activity of exogenous soil enzymes

Enzyme assays measure the maximum potential of enzymatic activity, not the realized rate (German et al., 2011). The activity of  $\beta$ -glucosidase (BG), acid phosphatase (AP) and leucine aminopeptidase (LAP) are assessed. The used substrates for enzymes are shown in Table 3.

Table 3: Incubation time and used substrates of enzymes (BG, AP and LAP) for soil activity

soil enzymes	substrate to indicate enzyme activity	incubation time
$\beta$ -glucosidase (BG)	4-methylumbelliferyl BD-gluco-pyranoside 6.77 mg/100 mL TRIS	2 hours
acid phosphatase (AP)	4-methylumbelliferylphosphate 5.12 mg/100 mL TRIS	1 hour + 3 hours
leucin aminopeptidase (LAP)	L-leucine-7-amido-4-methylcoumarinhydrochloride 6.5 mg/100 mL TRIS	24 hours

Soil bacteria and fungi populations produce extracellular enzymes (EEAs), which are involved in biochemical processes of soil. Fluorometric enzyme assays show the disparity of substrate fluorescence to quantify enzyme activity. Enzyme activity can be assessed exactly in the moment of exit of fluorescent dye from the substrate by an enzyme-catalysed reaction. The fluorescent rate gives information about the level of substrate degradation.  $\beta$ -glucosidase supports sugar degradation, acid phosphatase is important for phosphorus mineralization and leucin aminopeptidase reinforce protein degradation (Steinweg and McMahon, 2012).

Studying the catalysis function of enzymes in soils, 4-methylumbelliferone (MUF) offer a fast procedure to check soil biochemical activities of organic matter degradation and nutrient metabolism (Giacometti et al., 2014).

Buffer, soil samples, and standard MUF is prepared on the day of use. Standard MUF, substrate and soil samples are diluted with a 0.5 M TRIS buffer solution at pH 7.5 (6.057 g TRIS-base + 1L distilled water). Enzyme substrates is pre-dissolved in DMSO

(dimethyl sulfoxide) and 100 mL TRIS buffer is added (Table 3) to reach final concentration. Five different concentrations of 4-methylumbelliferone (MUF) standard solution are pre-prepared in TRIS buffer with concentrations of 0, 5, 10, 15, 20, 25  $\mu\text{M}$ . Firstly, standard curve is prepared and measured to check the accuracy of dilution series. Samples for standard curve are set up in flat-well black polystyrene 96-well microplates. 50  $\mu\text{L}$  diluted standard MUF + 200  $\mu\text{L}$  buffer (blank ve-water) or soil slurry are added to standard curve well-plate (German et al., 2011; Giacometti et al., 2014). For soil homogenization, 1 g of soil sample (Figure 8) is suspended in 125 ml 0.5 M TRIS buffer (pH 7.5), stirred with stir bar on stir plate for one minute, put in ultrasonic bath for 1 min and stirred again for 1 min. Samples for standard curve and enzyme assays are set up in flat-well black polystyrene 96-well microplates. Firstly, 200, 250 or 50  $\mu\text{L}$  buffer (Table 25), secondly, 200  $\mu\text{L}$  of sample soil slurries and finally enzyme substrate have to be added

Table 27) to assay well-plate. Prepared samples have to stay in darkness immediate, because enzymes are extremely sensible to light (German et al., 2011; Giacometti et al., 2014).



Figure 8: 1g of soil samples ready to put in 125 mL TRIS buffer, Mayer (2020)

Assay and standard plates are analysed after incubation time, as mentioned in Table 3. Microplates were covered and incubated in darkness at 30 °C. Fluorescence intensity is measured by a microplate fluorometer with 365 nm excitation filters, 450 nm emission filters and gain of 1150 (BG and AP) and gain of 920 for LAP. Rates of fluorescence rise are transferred into enzyme activity (nmol MUF/g/1h) (German et al., 2011).

#### 4.2.2 Gravimetric soil moisture content

Soil samples are obtained from 0-10 cm and 10-20 cm depth. A composite sample of four soil removals are taken. Soil samples are collected in labelled plastic bags and sealed. It is necessary to cool the soil during transport to the laboratory for further analyses. At the laboratory the soil of each sample is mixed, and plant debris or stones are removed. Afterwards 30 g of soil are weighted and packed in small paper bags. The soil samples are dried at 70 °C for one week. At back-weighing, water absorption from air must be prevented. The differences in weight of soil samples before and after drying results in measuring water loss as an indication of soil water content (in grams).

Vineyard cultivation and precipitation determines the date of sampling. The soil has to be moderate dry. Soil samples are sampled on April 6<sup>th</sup> and July 15<sup>th</sup> before soil cultivation. On May 4<sup>th</sup>, June 3<sup>rd</sup>.and July 30<sup>th</sup> soil samples are sampled after the cultivation of under-trellis area.

#### 4.2.3 Yield estimation per vine

The number of clusters per vine and yield as grapes weight per vine is quantified. The measurements are carried out using a hanging scale (accuracy  $\pm 10\%$ ) (Figure 9) directly in the vineyard. Figure 10 shows the method of harvesting every single vine separately by buckets. Grapes are harvested on September 19<sup>th</sup>.



Figure 9: Scale for weighing the stock weight at experimental vineyard, Mayer (2020)



Figure 10: Buckets to harvest every single vine separately, Mayer (2020)

#### **4.2.4 Fruit quality parameters**

Fruit quality like sugar content of grape is measured by refractometer. Further parameters of ripeness like total soluble solids (=density), tartaric acid, pH and NOPA (yeast usable nitrogen) of grape are assessed by fourier-transform infrared (FTIR) spectrometer of FOSS GesmbH. FTIR is an infrared spectroscopy technique, that measures vibration spectra of organic substances due to infrared radiation (Bauer et al., 2008). The samples are pressed, centrifuged, and filtered by filter paper to remove suspended solids. The prepared sample is filled in sample tubes to be analysed by the FTIR console. The grape must samples are measured two weeks before harvesting on September 3<sup>rd</sup> and two days before harvesting on September 17<sup>th</sup>.

#### **4.3 Statistical analyses**

Statistical analyses are conducted using R Studio version 1.1.453. The limit to reject the null hypothesis is set at  $\alpha = 0.05$ . Levels of significance are fixed at  $p > 0.05$  (not significant),  $p \leq 0.05$  \*,  $p \leq 0.01$  \*\*,  $p \leq 0.001$ \*\*\* (high significant.) The experimental results have been analysed by Levene's Test for Homogeneity of Variance. A p-value of  $>0,05$  indicates, that data is homogeneously distributed (not significant). For testing the data on normal distribution, a Shapiro Wilks test was performed. A correlation between seasonal precipitation (14-days before sampling) and soil moisture is checked by Pearson test. T-test, two-way and one-way ANOVA with subsequent post-hoc test according to Tukey are used to find significant differences. All figures are configured by SigmaPlot Version 14.0.3.192.

## 5 Results

### 5.1 Soil moisture

In order to relate the gravimetric soil water content to the climatic condition throughout the season, the sum of precipitation 14 days before the sampling timepoint are shown in Table 4. In addition for better illustration, on Figure 11 to Figure 15 the correlations of soil moisture and 14-days sum of precipitation before sampling of different treatments are shown. Soil moisture contents of under-trellis treatments in 0-10 cm (blue) and 10-20 cm (red) depth are compared.

Table 4 quotes the sum of precipitation of the last 14-days before date of sampling. In September high precipitation and a noticeable rise in temperature sum were recorded. There has been little rain in spring, with 0.8 mm in a period of 14-days. The highest precipitation was recorded before sampling on July 30<sup>th</sup> (54 mm).

*Table 4: Sum of precipitation in mm 14-days before sampling*

<b>sample dates of soil samples in 0-10 cm and 10-20 cm</b>	<b>sum of precipitation (mm) 14-days before sampling</b>
April 6 <sup>th</sup>	0.8
May 4 <sup>th</sup>	7.8
June 3 <sup>rd</sup>	22.4
July 15 <sup>th</sup>	16.8
July 30 <sup>th</sup>	54
September 22 <sup>nd</sup>	0

For all treatments, a correlation between soil moisture and precipitation is calculated. Only the treatment “Control” (R= 0,604) (Figure 11) and “UVW” (R=0,525) (Figure 15) showed a correlation on soil layer 0-10 cm. In 10-20 cm only the treatment “UVW” shows a light correlation (R= 0,488). None of the statistical analyses show correlations that could be considered as statistically significant.

In the following figures 14-16, the correlations for each treatment are presented.

Table 5 shows the R- and p-values of correlation between soil moisture and precipitation 14 days before sampling at different soil layers.

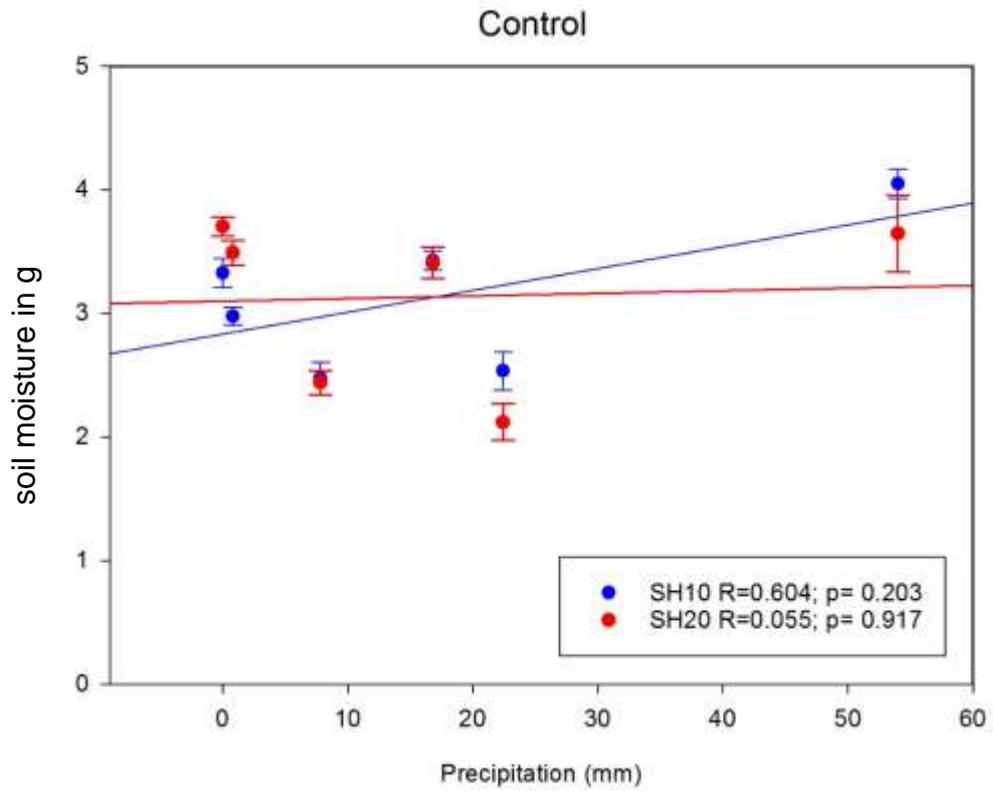


Figure 11: Correlation of soil moisture and 14-days sum of precipitation in mm before sampling in treatment "Control"

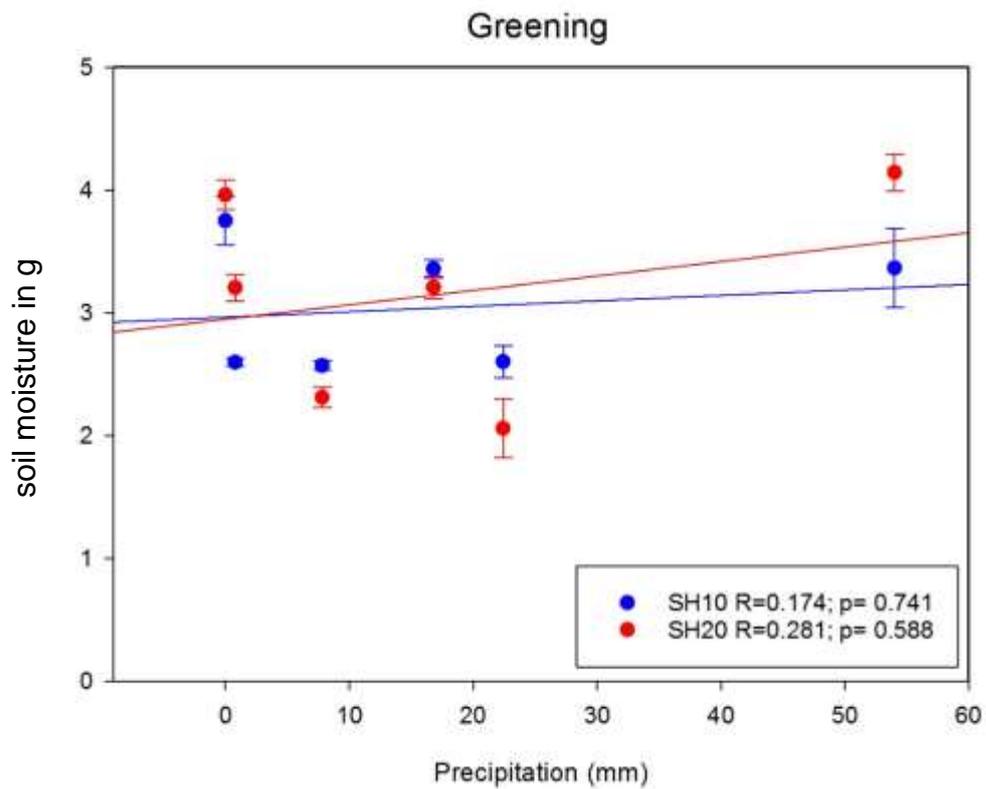


Figure 12: Correlation of soil moisture and 14-days sum of precipitation in mm before sampling in treatment „Greening”

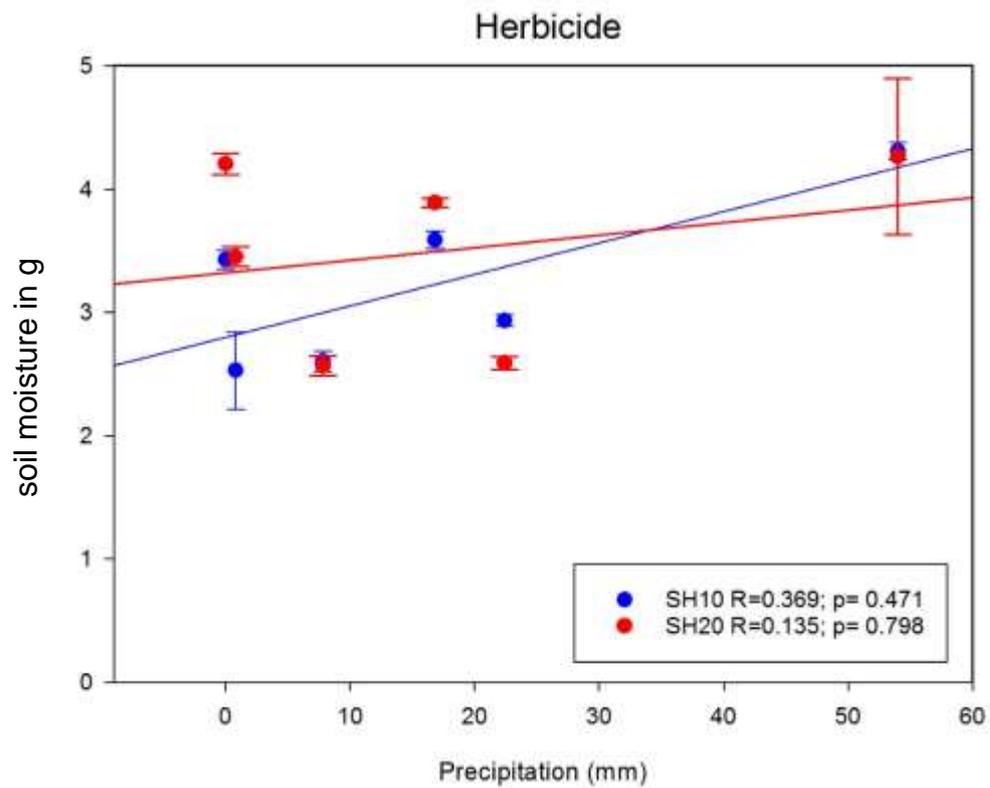


Figure 13: Correlation of soil moisture and 14-days sum of precipitation in mm before sampling in treatment „Herbicide”

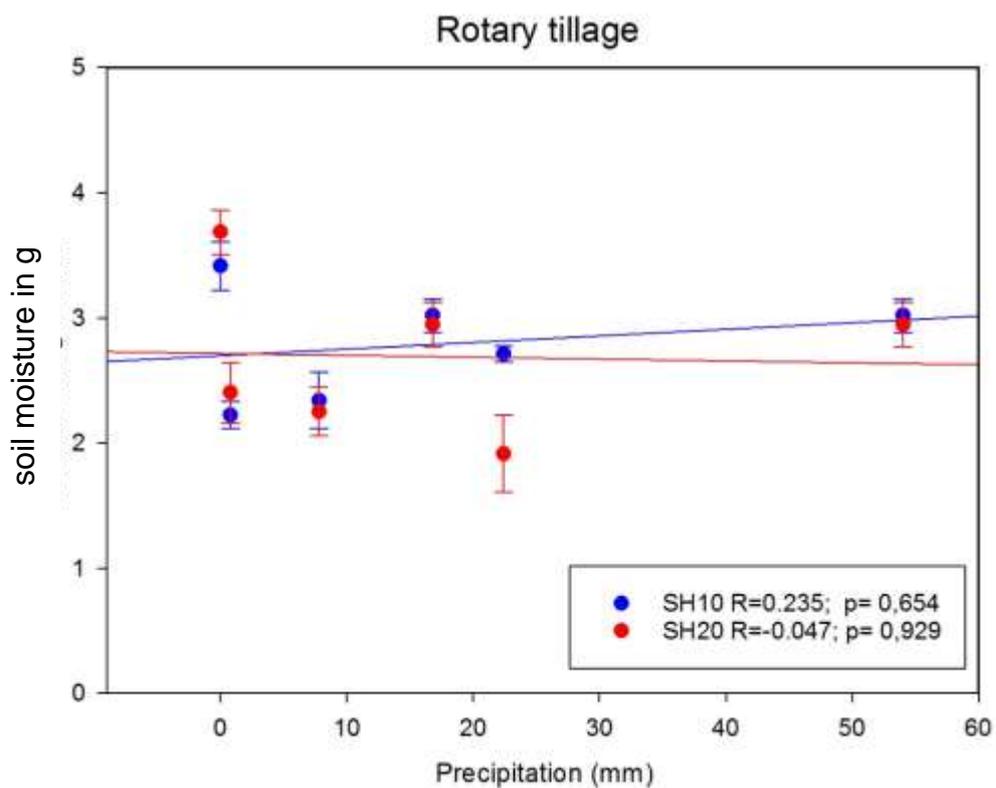


Figure 14: Correlation of soil moisture and 14-days sum of precipitation in mm before sampling in treatment „Rotary tillage”

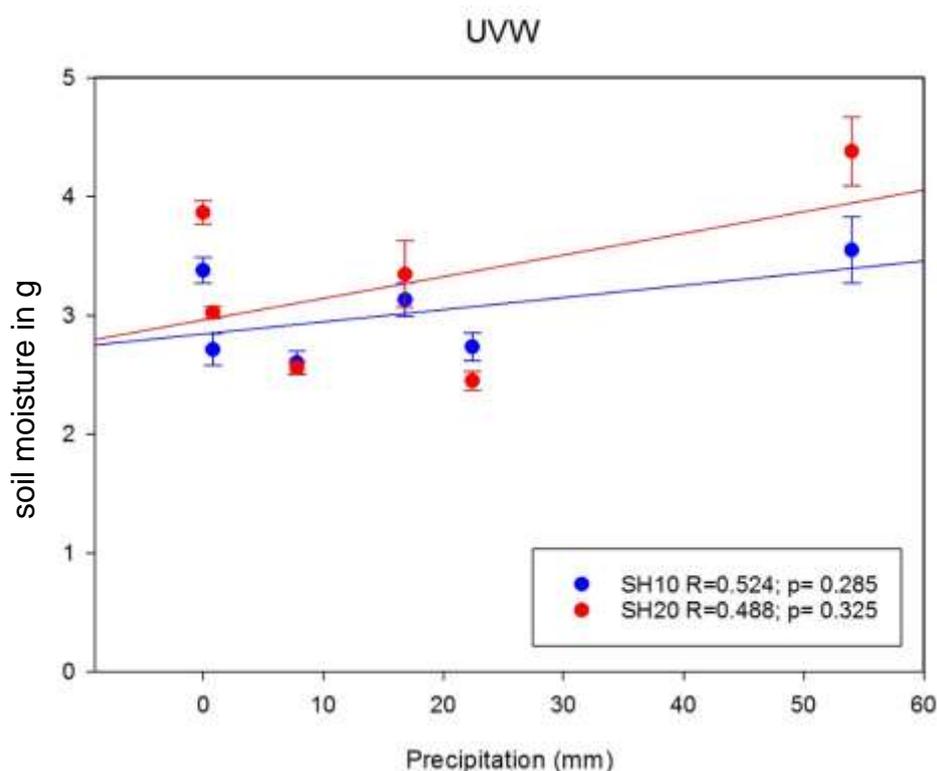


Figure 15: Correlation of soil moisture and 14-days sum of precipitation in mm before sampling in treatment „Under vine weeler”

Table 5: R-value and p-value of soil moisture in correlation to precipitation (sum of precipitation 14-days before sampling) of different under-trellis treatments in 0-10 cm (SH10) and 10-20 cm (SH20) layers

under-trellis treatment	R-value (SH10)	p-value (SH10)	R-value (SH20)	p-value (SH20)
Control	0.604	0.204	0.055	0.917
Greening	0.174	0.724	0.282	0.589
Herbicide	0.369	0.471	0.135	0.798
Rotary tillage	0.235	0.654	0.047	0.929
UVW	0.525	0.285	0.488	0.326

The influence of tillage and sampling date on the soil moisture content SH10 and SH20 is analyzed by a two-factor ANOVA. The statistical results are shown in (Table 6) while the effect plots are shown in Figure 11 to Figure 15. Soil moisture in 10-20 cm ( $<0.001^{***}$ ) is highly significant and in 0-10 cm ( $0.004^{**}$ ) significant effected by under-trellis strategy (Table 6). The date has a strong effect on soil moisture (SH10:  $<0.001^{***}$ , SH20:  $<0.001^{**}$ ).

Table 6: p-value of ANOVA: influence of tillage and date on soil moisture in 0-10 cm (SH10) and 10-20 cm (SH20)

	p-value of ANOVA	
	SH10 (0-10 cm)	SH20 (10-20 cm)
tillage	0.004**	<0.001***
date	<0.001***	<0.001***
tillage*date	0.621	0.767

Figure 16 and Figure 17 show the differences in soil moisture depending on soil depth (0-10 cm and 10-20 cm) of the treatments. Table 7 lists the totalled mean values of soil moisture of all sampling dates and percentage of soil moisture loss of the different treatments compared to the highest value. In 0-10 cm and 10-20 cm soil moisture of treatment “Herbicide” is assumed to be 100 %, because treatment “Herbicide” shows the highest soil moisture values of 3.23 g (SH10) and 3.49 g (SH20). The lowest soil moisture occurs in treatment “Rotary tillage” regardless of soil depth. The mean values (Table 7) of all sampling dates of treatment “Rotary tillage” are 2.87 g (SH10) and 2.61 g (SH20). In layer 10-20 cm of treatment “Rotary tillage” a 25.35 % loss of soil moisture is measured. Compared to the other treatments the treatment “Control” has a higher loss of soil moisture in 10-20 cm (-10.35 %) than in 0-10 cm (-3.12 %). On the last sampling date (September 22<sup>nd</sup>) soil moisture of all treatments show the least difference (Figure 16) in 0-10 cm. In Figure 17 a remarkable difference in soil moisture of rotary tillage compared to the other treatments, on July 30<sup>th</sup> (SH20: 2.95 g) is notable.

Table 7: Mean values in nmol/g soil\*hour of soil moisture concerning all sampling dates in 0-10 cm (SH10) and 10-20 cm (SH20) layers and loss of soil moisture in %

under-trellis treatment	mean value of soil moisture (SH10) 0-10 cm	loss of soil moisture in %	mean value of soil moisture (SH20) 10-20 cm	loss of soil moisture in %
Control	3.13	-3.12	3.13	-10.35
Greening	3.04	-5.89	3.15	-9.89
Herbicide	3.23		3.49	
Rotary tillage	2.87	-11.23	2.61	-25.35
UVW	3.02	-6.62	3.27	-6.40

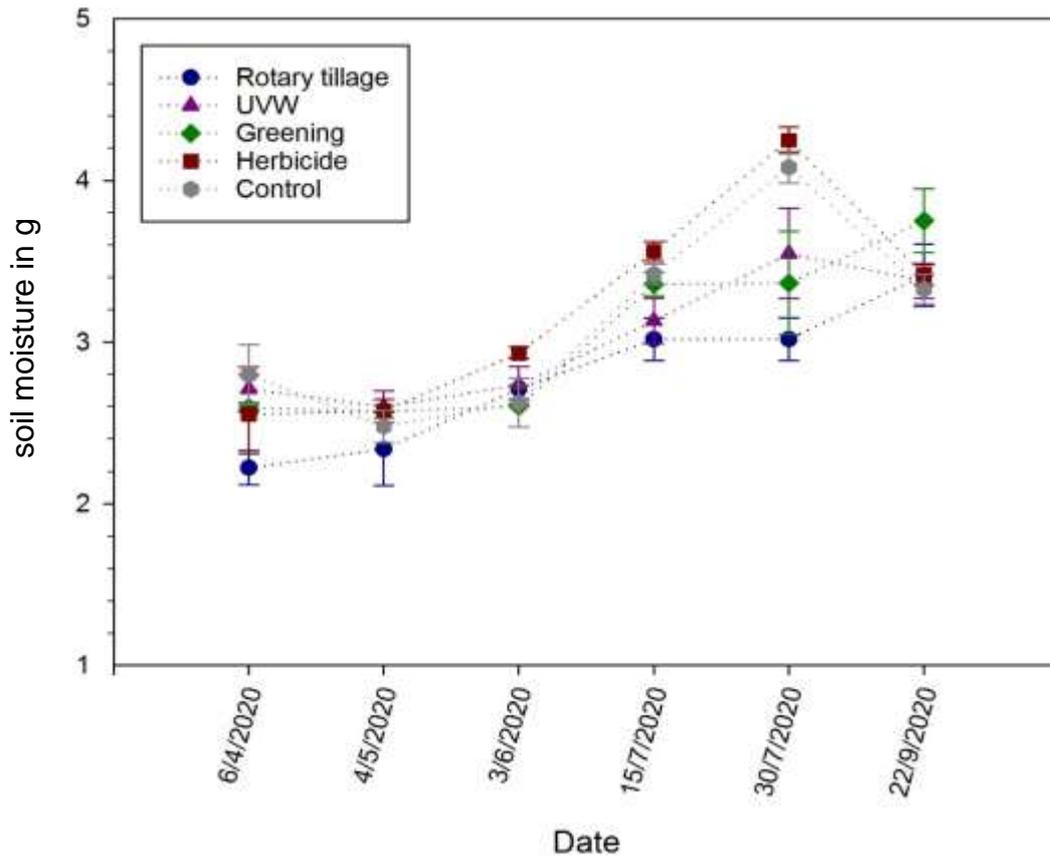


Figure 16: Effects of different under-trellis treatments on soil moisture in 0-10 cm (mean  $\pm$  standard error)

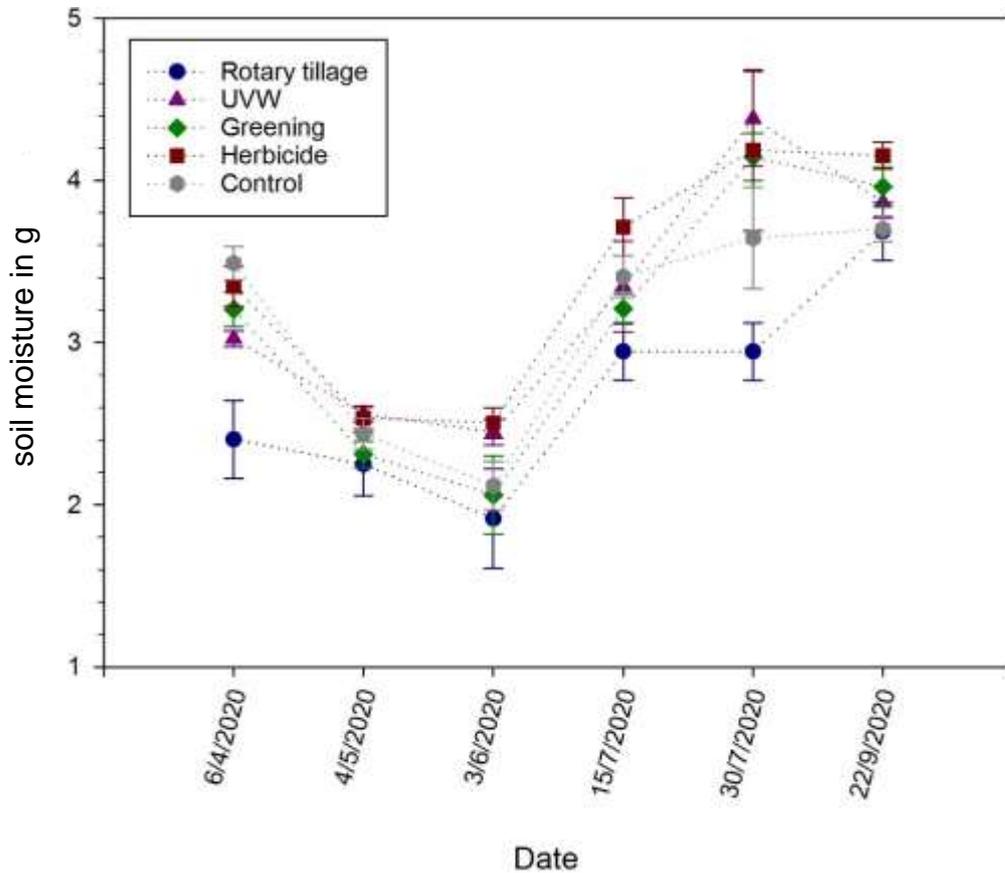


Figure 17: Effects of different under-trellis treatments on soil moisture (10-20 cm) subjected to date (mean  $\pm$  standard error)

The post-hoc test in Table 8 compares soil moisture of under-trellis strategies of different soil layers and on all sampling dates. The results show a significant difference in soil moisture between “Rotary tillage” and “Herbicide” in 10-20 cm of all sample dates (p-value: 0.004\*\*).

Table 8: P-values of post-hoc test comparing soil moisture of under-trellis strategies in 0-10 cm (SH10) and 10-20 cm (SH20)

<b>under-trellis treatments</b>	<b>p-value in 0-10 cm (SH10)</b>	<b>p-value in 10-20 cm (SH20)</b>
herbicide – greening	0.770	0.532
control – greening	0.982	0.999
rotary tillage – greening	0.527	0.246
UVW – greening	0.999	0.982
control – herbicide	0.972	0.486
rotary tillage – herbicide	0.056	0.004**
UVW – herbicide	0.685	0.853
rotary tillage – control	0.224	0.280
UVW – control	0.957	0.972
UVW – rotary tillage	0.620	0.076

The application of a grass mulch layer shows similar trends on all treatments (Figure 18 and Figure 19). UVW treatment with mulch layer show the lowest soil moisture on all sampling dates and in both sampling layers (SH10: 4.14 g and SH20: 4.09 g). “Herbicide+mulch” (SH10: 4.90 g and SH20: 4.69 g) and “Greening+mulch” (SH10: 4.86 g and SH20: 4.71 g) treatments show roughly equal results at the end of vegetation period (September 22<sup>nd</sup>). On July 15<sup>th</sup> the under-trellis treatment “Herbicide+mulch” (SH10: 4.56 g and SH20: 4.47 g) has more soil moisture than “Greening+mulch” (SH10: 4.31 g and SH20: 4.12 g) treatment. At the second sampling date (July 30<sup>th</sup>) the results reversed. The results show higher soil moisture values in the "Greening+mulch" (SH10: 4.52 g and SH20: 4.44 g) than in the "Herbicide" (SH10: 4.16 g and SH20: 4.36 g) variant.

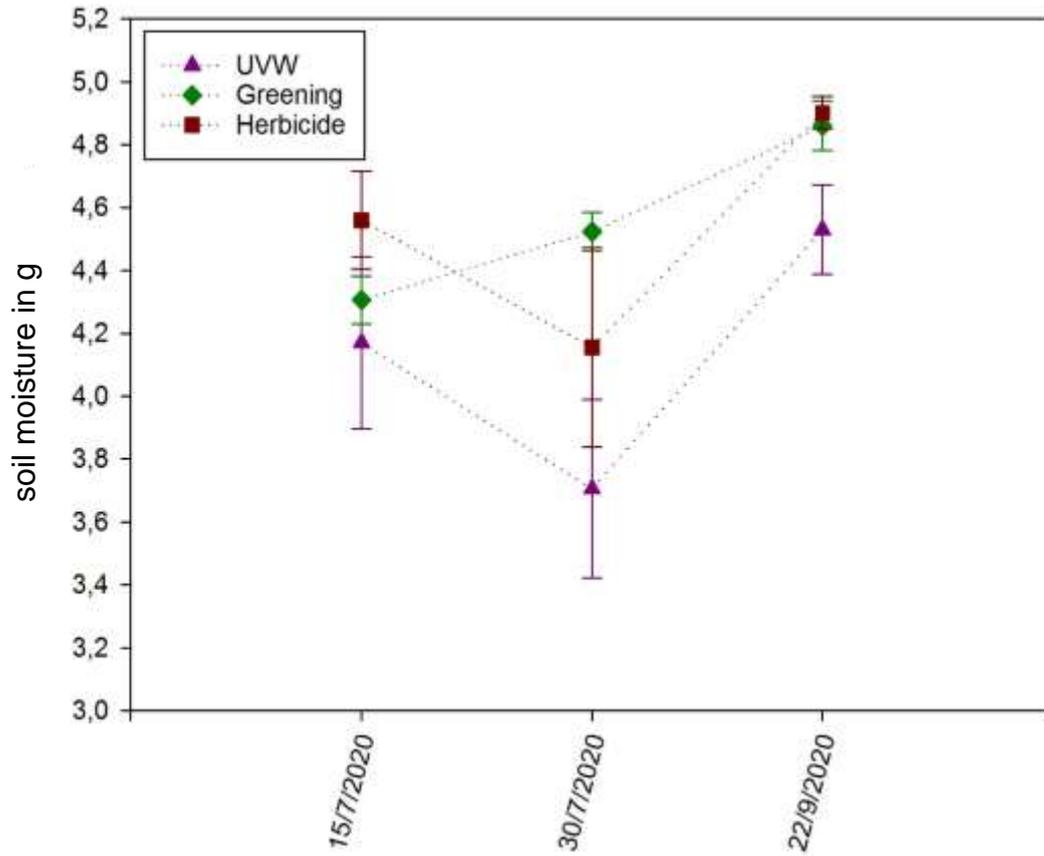


Figure 18: Soil moisture of treatment “UVW”, “Greening” and “Herbicide” with a grass mulch layer in 0-10 cm subjected to sample date (mean  $\pm$  standard error)

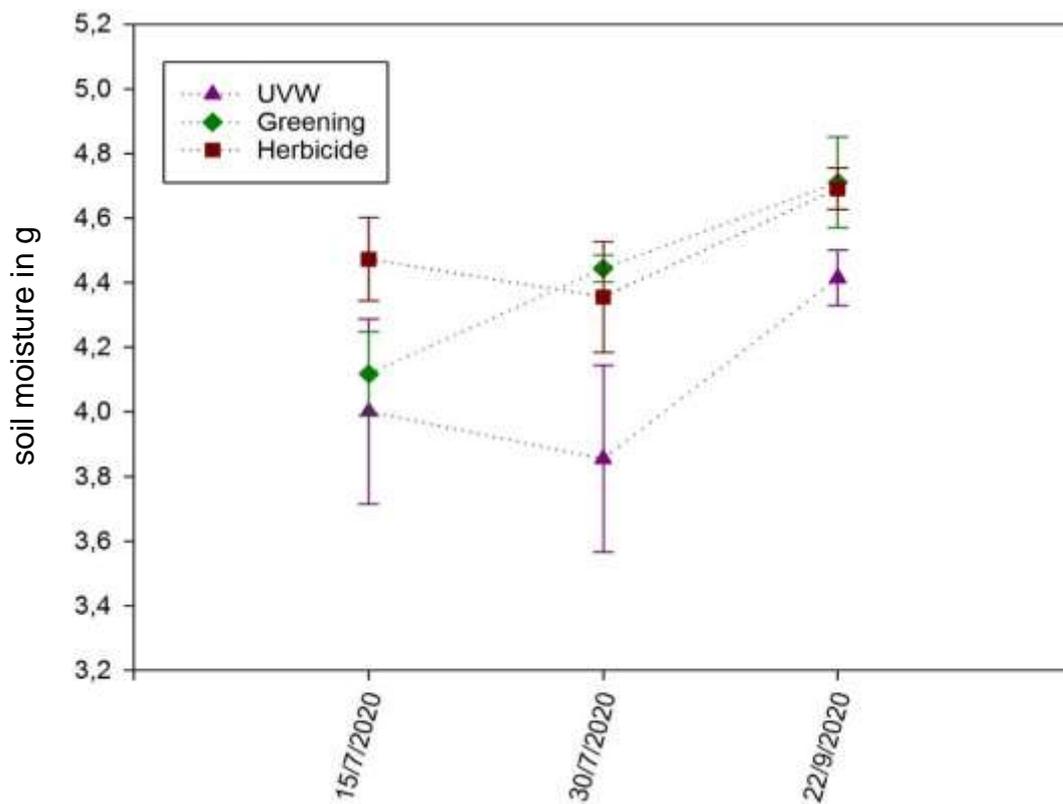


Figure 19: Soil moisture of treatment “UVW”, “Greening” and “Herbicide” with a grass mulch layer in 10-20 cm subjected to sample date (mean  $\pm$  standard error)

The t-test in Table 9 compares Herbicide, Greening and UVW standard treatment with corresponding grass mulch layer treatment. Table 9 points out that a strong significant difference between standard treatments and mulch layer in 0-10 cm occurs (<0.001\*\*\*). Only the Herbicide treatment (standard compared to mulch) shows a significant difference in soil moisture in 10-20 cm depth. Differences in soil moisture in 10-20 cm sampling depth are significant in “Herbicide“ and “Greening” treatments.

Table 9: P-values of t-test comparing soil moisture of standard under-trellis strategies (UVW, Herbicide, Greening) and a mulch layer treatment (Herbicide+mulch, UVW+mulch, Greening+mulch) in 0-10 cm (SH10) and 10-20 cm (SH20)

under-trellis treatments	p-values of soil moisture (t-test)	
	0-10 cm (SH10)	10-20 cm (SH20)
Herbicide+mulch – Herbicide	<0.001***	0.004**
UVW+mulch – UVW	<0.001***	0.334
Greening+mulch – Greening	<0.001***	0.009**

Table 10 and Table 11 show, that the application of a grass mulch layer rises soil moisture of all treatments in both soil layers. Most influence of the grass mulch layer is seen on the treatment “Greening”. The soil moisture increases by 50.05 % in 0-10 cm and 40.5 % in 10-20 cm. The treatments “UVW” (SH10: 3.15 g and SH20: 3.12 g) and “UVW+mulch” (SH10: 4.14 g and SH20: 4.09 g) show the lowest capacity to store soil moisture. The increase of soil moisture caused by the application of a grass mulch layer is only 37.04 % in 0-10 cm and 25.06 % in 10-20 cm of treatment “UVW”.

Table 10: Mean values (nmol/g soil\*hour and all sampling dates) of soil moisture in 0-10 cm of under-trellis strategies (UVW, Greening, Herbicide) with and without a grass mulch layer and the change of soil moisture in % in the individual strategies

under-trellis strategies	mean value of standard treatment (no mulch layer)	mean value of treatments with a grass mulch layer	change of soil moisture in % (standard vs. grass mulch layer)
UVW	3.15	4.14	37.04
Greening	3.04	4.56	50.05
Herbicide	3.23	4.54	40.48

## Results

Table 11: Mean values (nmol/g soil\*hour and all sampling dates) of soil moisture in 10-20 cm of under-trellis strategies (UVW, Greening, Herbicide) with and without a grass mulch layer and the change of soil moisture in % in the individual strategies

under-trellis treatment	mean value of standard treatment (no mulch layer)	mean value of treatments with a grass mulch layer	change of soil moisture in % (standard vs. gras mulch layer)
UVW	3.27	4.09	25.06
Greening	3.15	4.42	40.51
Herbicide	3.49	4.51	28.98

Figure 20 and Figure 21 clarifies the differences between the standard under-trellis treatments “Greening”, “UVW” and “Herbicide” with and without grass mulch layer. The content of soil moisture under the grass mulch layer increases in all three treatments, except the treatment “Herbicide” in 0-10 cm and “UVW” in 10-20 cm on July 30<sup>th</sup>.

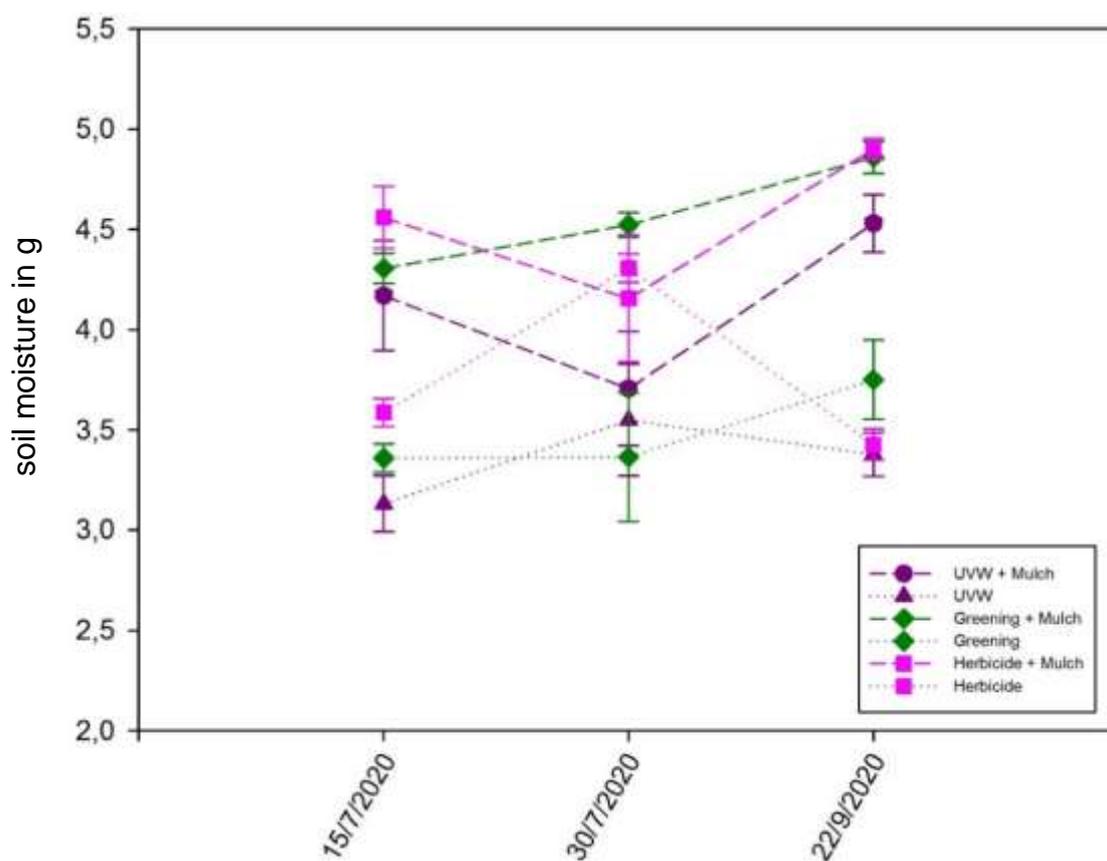


Figure 20: Comparison of UVW, Herbicide and Greening treatments with and without grass mulch layer by soil moisture in 0–10 cm

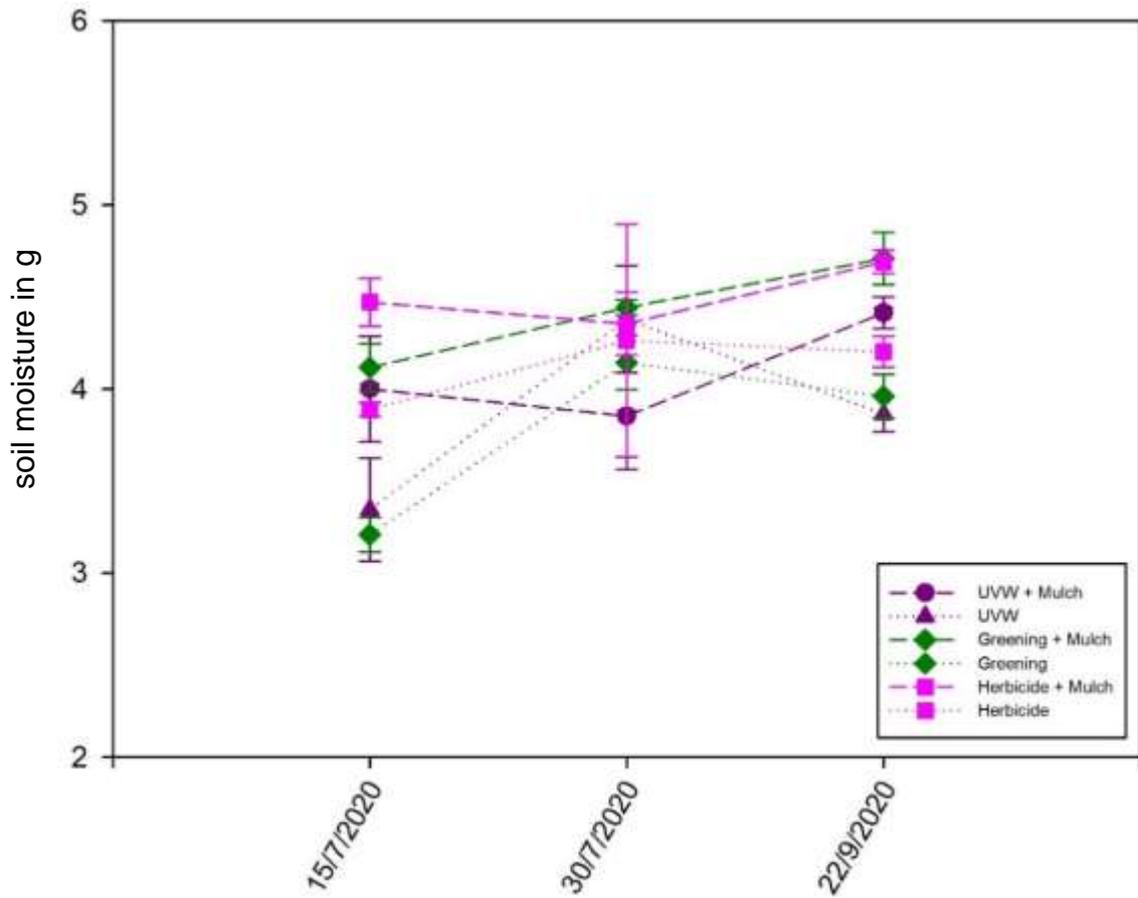


Figure 21: Comparison of UVW, Herbicide and Greening treatments with and without grass mulch layer by soil moisture in 10-20cm

## 5.2 Activity of soil enzymes

The influence of four different under-trellis strategies on the enzyme activity of acid phosphatase after three-hours of incubation time on five sampling dates (April 6<sup>th</sup>, June 3<sup>rd</sup>, July 14<sup>th</sup>, July 30<sup>th</sup> and September 22<sup>th</sup>) are shown in Figure 22. With one exception, the highest enzyme activity of AP is recognizable in the treatment “Control” (155, 142, 140, 148, 150 nmol/g soil\*hour) and “Greening” (140, 124, 137, 135, 137 nmol/g soil\*hour). Just once, on June 6<sup>th</sup>, the highest enzyme activity of AP (144 nmol/g soil\*hour) is recorded in the treatment “Rotary tillage”.

On July 30<sup>th</sup>, a remarkable decrease of AP activity is recorded in the treatment “Herbicide”. Generally, the enzyme activity of acid phosphatase remains stable during vegetation period. Enzyme activity of acid phosphates under grass mulch layer and an incubation time of three hours (Figure 23) has been sampled on three dates in the treatments “Greening”, “Herbicide” and “UVW “. Generally, the highest AP activity takes place in treatment “Greening” (150, 164, 175 nmol/g soil\*hour). In all three treatments an increase of AP activity during vegetation period is apparent.

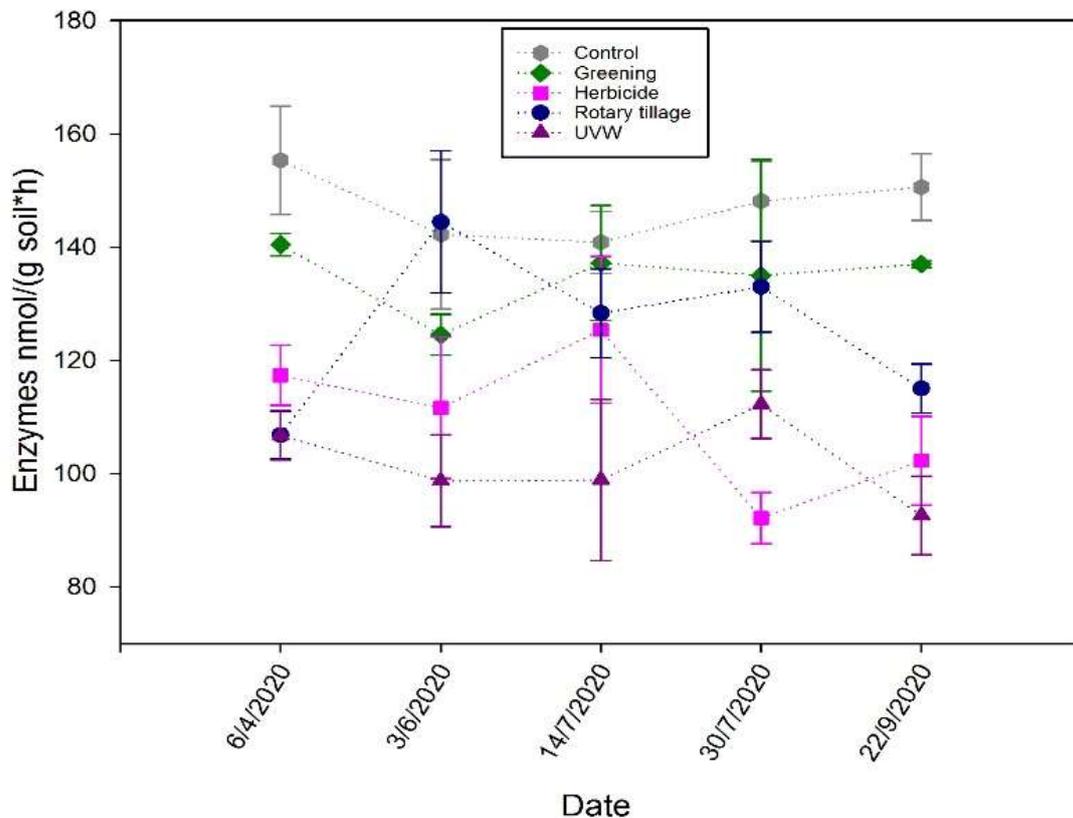


Figure 22: Enzyme activity of acid phosphatase after 3-hours incubation time on the five different under-trellis treatments (Control, Greening, Herbicide, Rotary tillage, Control) without a mulch layer

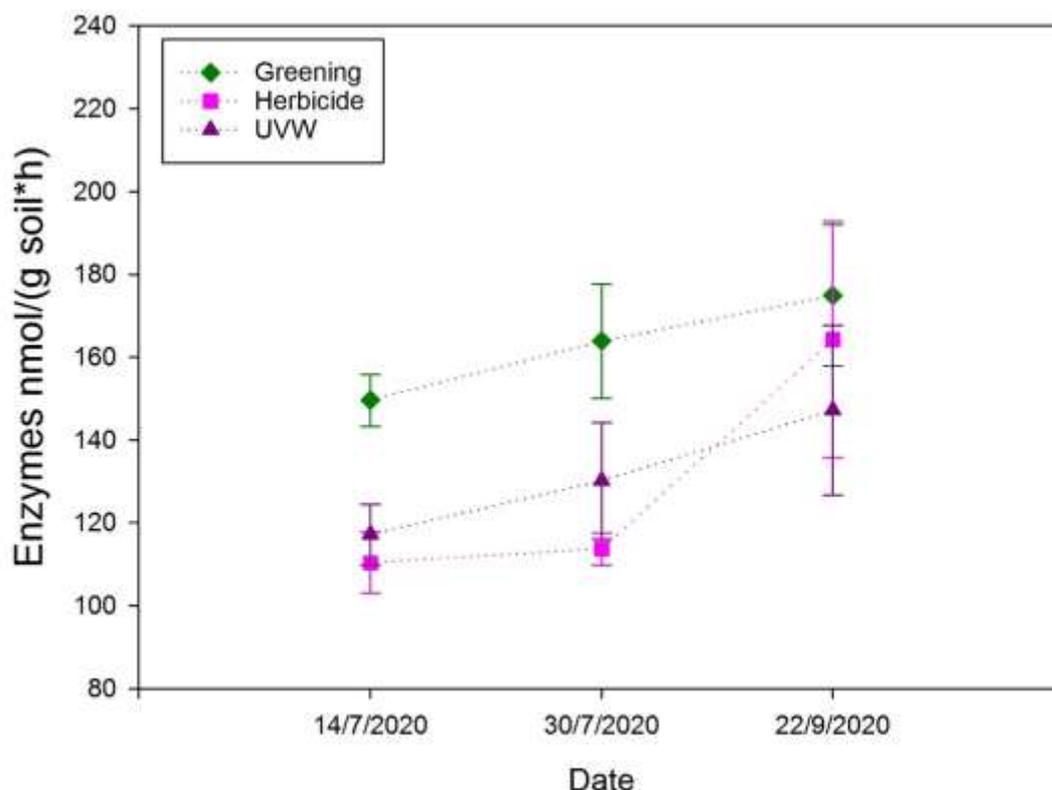


Figure 23: Enzyme activity of AP after 3-hours incubation time on UVW, Herbicide and Greening treatments under a grass mulch layer

The highest enzyme activity of AP is recorded in the treatment “Control”. The enzyme activity of “UVW” is reduced by 30.9 % compared to the “Control” treatment (Table 12).

Table 12: Interpretation and ranking of AP activity (nmol/(g soil\*hour)) in all standard treatments, treatment “Control” is taken as reference

ranking and activity of acid phosphatase (AP)			
ranking	treatment	mean value of enzyme activity (EA) in nmol/(g soil*hour)	decrease of EA in %
1	Control	147	
2	Greening	135	8.54
3	Rotary	126	14.85
4	Herbicide	110	25.54
5	UVW	102	30.90

On Figure 29 the activity on six sampling dates (April 6<sup>th</sup>, May 4<sup>th</sup>, June 3<sup>rd</sup>, July 14<sup>th</sup>, July 30<sup>th</sup>, September 22<sup>th</sup>) of the  $\beta$ -glucosidase enzyme after two-hours incubation time, subjected to under-trellis strategies, is shown. Enzyme activity of  $\beta$ -glucosidase

(BG) decreases during vegetation in all treatments. On all sample dates, the highest enzyme activity of BG occurs in the treatments “Control” (225, 164, 144, 116, 156, 130 nmol/g soil\* hour) and “Greening” (185, 167, 152, 117, 132, 119 nmol/g soil\* hour). On five of six sample dates the treatment “UVW” (148, 96, 89, 86, 78 nmol/g soil\*hour) shows the lowest enzyme activity of BG, only on July 30<sup>th</sup> the lowest enzyme activity can be seen in the treatment “Herbicide” (81 nmol/g soil\*hour).

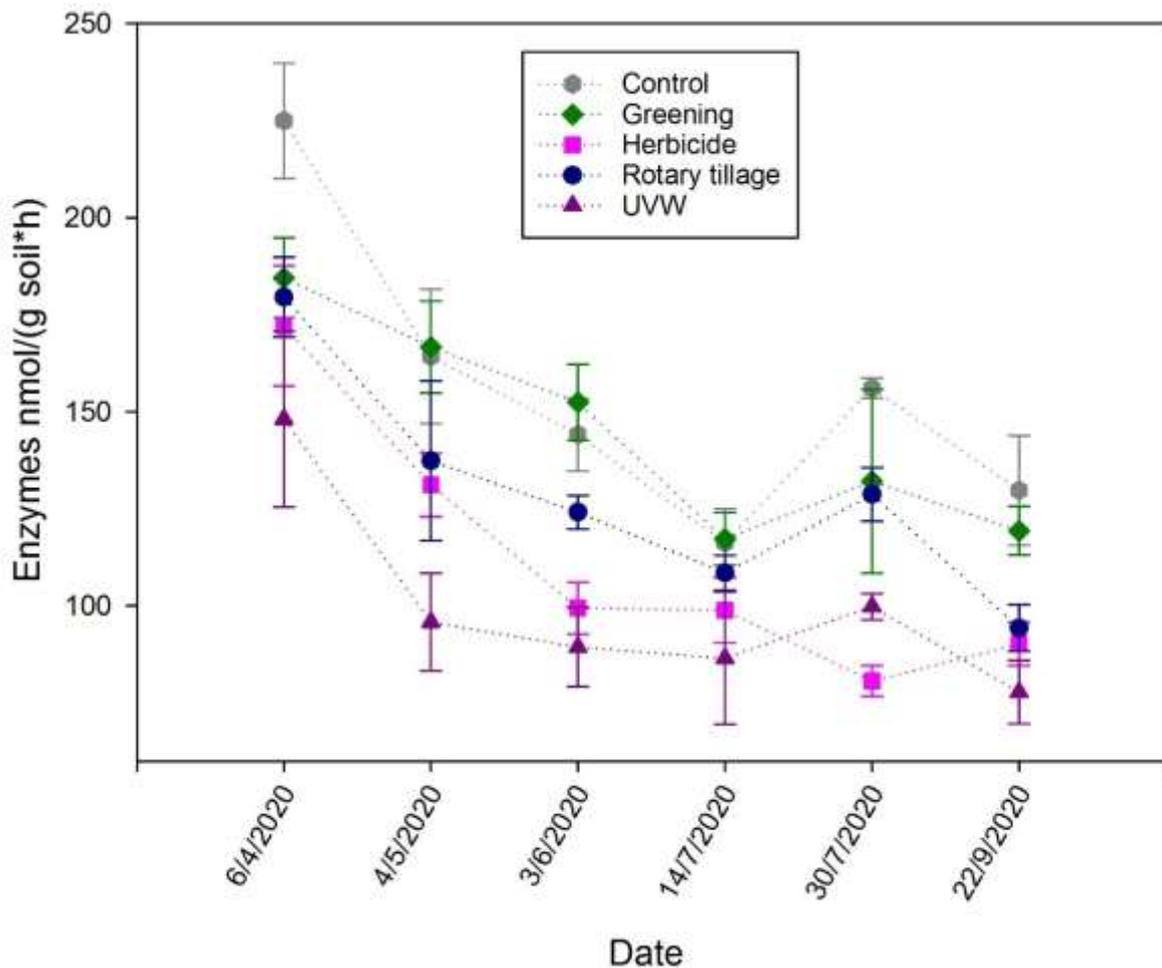


Figure 24: Enzyme activity of  $\beta$ -glucosidase after 2-hours incubation time on all sampling dates and standard treatments

Figure 25 depicts the activity of  $\beta$ -glucosidase enzyme after two-hours of incubation time with grass mulch layer in the treatments “Greening”, “Herbicide” and “UVW“. Generally, the highest BG activity takes place in treatment “Greening” (121, 124, 146 nmol/g soil\*hour), followed by treatment “UVW” (96, 110, 127 nmol/g soil\*hour) and as a taillight treatment “Herbicide” (90, 93, 122 nmol/g soil\*hour). In all three treatments an increase of BG activity during vegetation period is detectable.

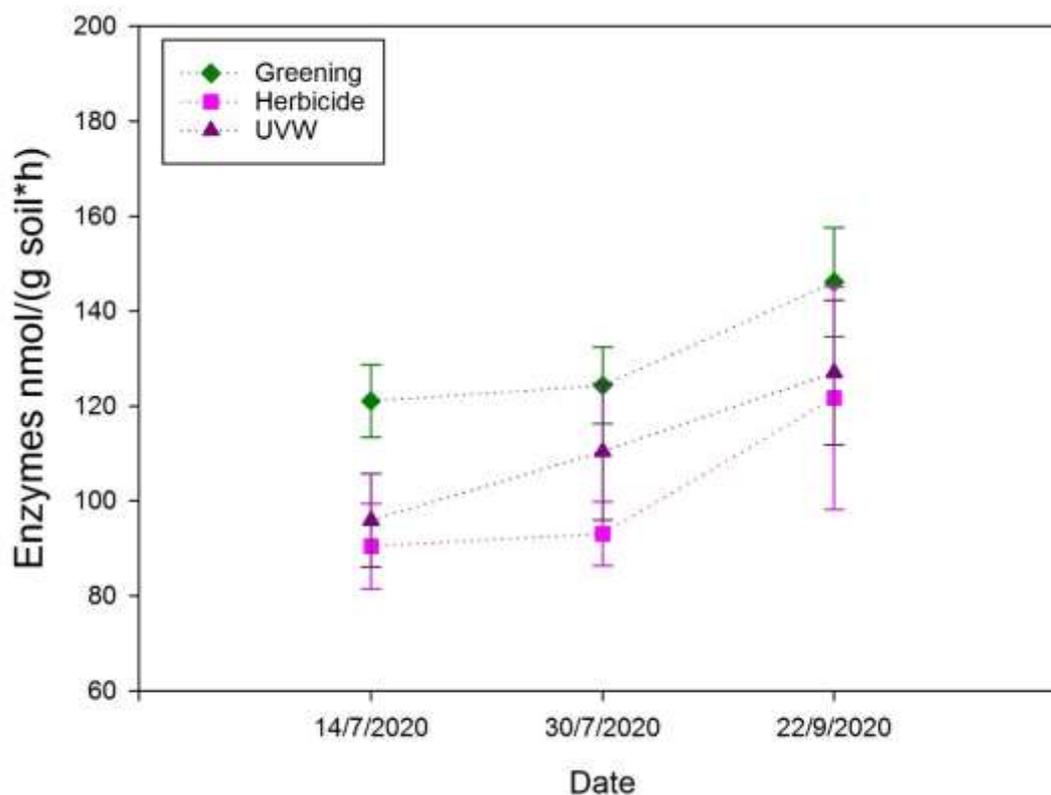


Figure 25: Enzyme activity in nmol/(g soil\*h) of  $\beta$ -glucosidase after 2-hours incubation time under grass mulch layer on July 7<sup>th</sup>, July 30<sup>th</sup> and September 22<sup>nd</sup> in 2020

The highest enzyme activity of BG is recorded in the treatment “Control”. The enzyme activity of UVW is reduced by 36.13 % compared to the “Control” treatment (Table 13).

Table 13: Interpretation and ranking of BG activity (nmol/(g soil\*hour)) in all standard treatments, treatment “Control” is taken as reference

ranking and activity of $\beta$ -glucosidase (BG)			
ranking	treatment	mean value of enzyme activity (EA) in nmol/(g soil*hour)	decrease of EA in %
1	Control	156	
2	Greening	145	6.74
3	Rotary	129	17.42
4	Herbicide	112	28.13
5	UVW	100	36.13

On Figure 26 the enzyme activity of leucine aminopeptidase (LAP) after 24-hours incubation time is shown. Most of the enzyme activity of LAP is notable in the treatment “Greening” (216, 235, 177, 213, 190 nmol/g soil\*hour) and the lowest LAP activity rates appear in the treatment “Herbicide” (187, 129, 164, 150, 170 nmol/g soil\*hour). The mechanical tillage variants are in the middle range.

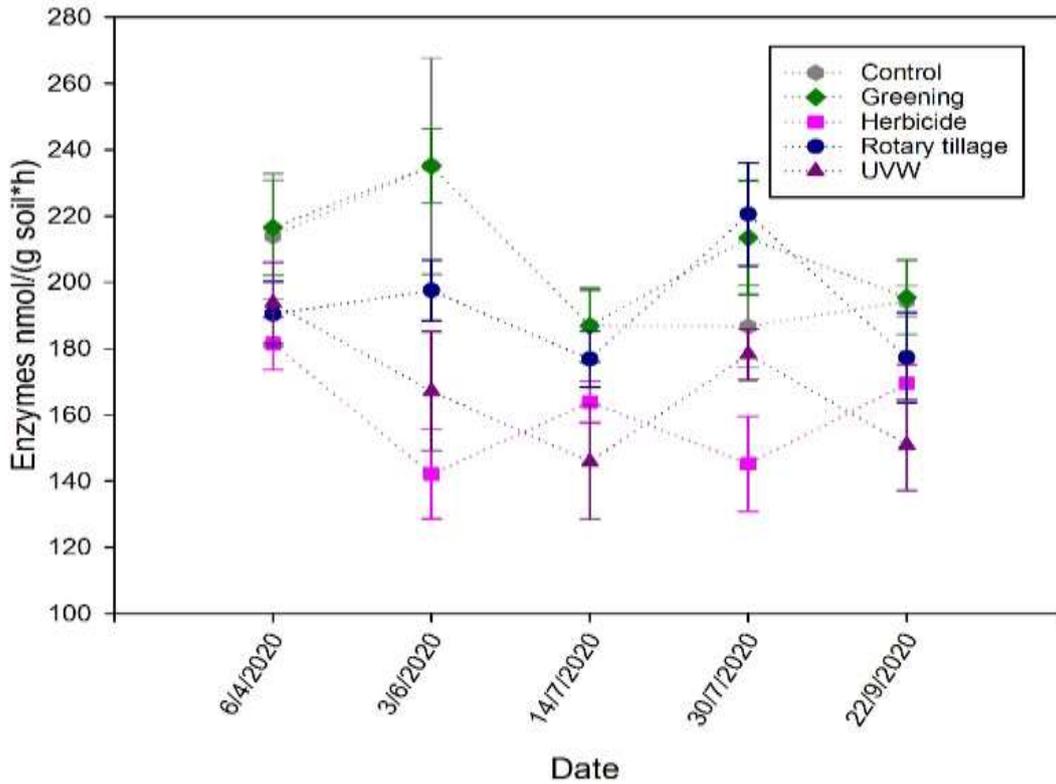


Figure 26: Enzyme activity in nmol/(g soil\*h) of leucine aminopeptidase after 24- hours incubation time on all sampling dates

Enzyme activity of leucine aminopeptidase after 24-hours of incubation time under grass mulch layer (Figure 27) shows the highest values in the treatment “Greening” (239, 207, 282 nmol/g soil\*hour) on all three sampling. In all three treatments the enzyme activity of LAP decreases from July 14<sup>th</sup> to July 30<sup>th</sup> and remarkable rises until September 22<sup>nd</sup>.

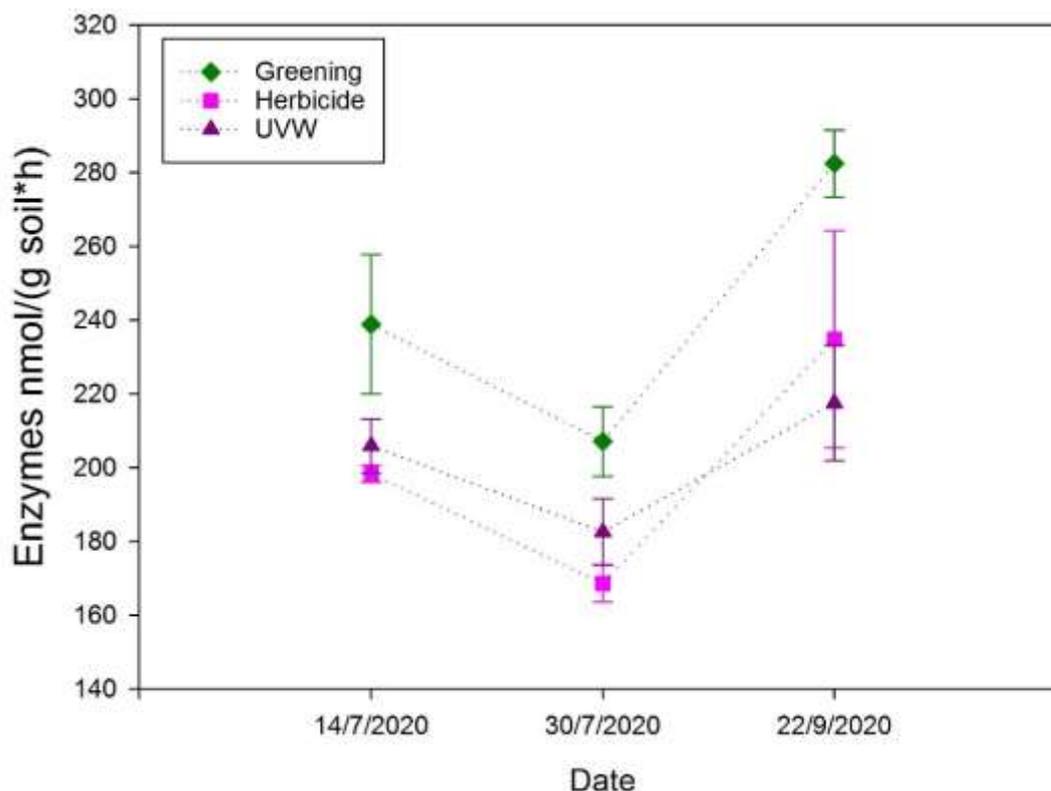


Figure 27: Enzyme activity of leucine aminopeptidase after 24-hours incubation time under grass mulch layer on July 7<sup>th</sup>, July 30<sup>th</sup> and September 22<sup>nd</sup> in 2020

The highest enzyme activity of BG can be recorded in the treatment “Control”. The enzyme activity of the treatment “Herbicide” is reduced by 22.93 % compared to the “Control” treatment (Table 13).

Table 14: Interpretation and ranking of LAP activity (nmol/(g soil\*hour)) in all standard treatments, treatment “Control” is taken as reference

ranking and activity of leucine aminopeptidase (LAP)			
ranking	treatment	mean value of enzyme activity (EA) in nmol/(g soil*hour)	decrease of EA in %
1	Control	208	
2	Greening	206	0.58
3	Rotary	189	8.97
4	Herbicide	170	18.15
5	UVW	160	22.93

Basically, the enzyme activity of AP increases after applying a grass mulch layer on greening treatment by 20.8 % from 135 to 163 nmol/g soil\*hour, in the herbicide treatment by 17,9 % from 110 to 129 nmol/g soil\*hour and in the “UVW” treatment by 29,1 % from 102 to 132 nmol/g soil\*hour. Enzyme activity of  $\beta$ -glucosidase decreases

under a grass mulch layer in all three treatments. After applying a grass mulch layer enzyme activity of LAP increases in the Greening treatment by 17.7 % from 206 to 243 nmol/g soil\*hour, in the Herbicide treatment by 25,4 % from 160 to 201 nmol/g soil\*hour and in the UVW treatment by 18,9 % from 170 to 202 nmol/g soil\*hour.

Distributed over all sampling dates the highest enzyme activities of acid phosphatase (Figure 22),  $\beta$ -glucosidase (Figure 24) and leucine aminopeptidase (Figure 26) in the standard treatments are apparent in the under-trellis treatments “Greening” and “Control”. In the “Greening” treatment the mean value (MV) of enzyme activity of acid phosphatase is 135,  $\beta$ -glucosidase is 146 and leucine aminopeptidase is 206 nmol/g soil\*hour. In the “Control” treatment the mean value (MV) of enzyme activity of acid phosphatase is 147,  $\beta$ -glucosidase is 156 and leucine aminopeptidase is 208 nmol/g soil\*hour. The mean value of enzyme activity of acid phosphatase (MV=102 nmol/g soil\*hour) and  $\beta$ -glucosidase (MV=100 nmol/g soil\*hour) in the treatment “UVW” and of leucine aminopeptidase (MV=160 nmol/g soil\*hour) in the treatment “Herbicide” bring up the rear.

Enzyme activities of acid phosphates (Figure 23),  $\beta$ -glucosidase (Figure 25) and leucine aminopeptidase (Figure 27) under grass mulch layer show the highest values of activity in the greening treatment (MV of AP: 163, MV of BG: 131 and MV LAP: 243 nmol/g soil\*hour). The herbicide treatment under a grass mulch layer notes the lowest enzyme activities with a mean value of AP: 129, BG: 102 and LAP: 201 nmol/g soil\*hour. High significant effects of under-trellis strategy on enzyme activity of all three tested enzymes (p-value: <0.001\*\*) are identifiable. The date has a strong effect on  $\beta$ -glucosidase (p-value: <0.001\*\*) and leucine aminopeptidase (p-value: 0.009\*\*). Table 15 shows the p-values of the conducted ANOVA.

Table 15: P-value of ANOVA for influence of tillage strategy and date on enzyme activity in 0-10 cm soil depth

	p-value of ANOVA of enzyme activity in 0-10 cm		
	acid phosphatase	$\beta$ -glucosidase	leucine aminopeptidase
tillage	<0.001***	<0.001***	<0.001***
date	0.297	<0.001***	0.009**
tillage*date	0.851	0.787	0.757

The values of the post-hoc test in Table 16 shows a high significant difference between some of the under-trellis treatments. The comparison of Rotary tillage with Greening, Greening with Control and UVW with Herbicide do not show a significant difference.

Table 16: P-values of post-hoc tests comparing under-trellis strategies in 0-10 cm (SH10) with enzyme activity of acid phosphatase (AP),  $\beta$ -glucosidase (BG) and leucine aminopeptidase (LAP)

<b>p-value of post-hoc tests (0-10 cm) of enzyme activity</b>			
<b>compared treatments</b>	<b>p-value (AP)</b>	<b>p-value (BG)</b>	<b>p-value (LAP)</b>
Greening – Control	0.210	0.854	0.968
Herbicide – Control	<0.001***	<0.001***	<0.001***
Rotary tillage – Control	0.006**	0.008**	0.800
UVW – Control	<0.001***	<0.001***	0.002**
herbicide - greening	<0.001***	0.024*	<0.001***
Rotary tillage – Greening	0.629	0.499	0.413
UVW – greening	<0.001***	<0.001***	<0.001***
Rotary tillage – Herbicide	0.049*	0.589	0.011*
UVW – Herbicide	0.662	0.688	0.956
UVW – rotary tillage	<0.001***	0.049*	0.072

Table 17 shows the increase of AP and LAP activity caused by the application of a grass mulch layer. The enzyme activity (EA) of AP and LAP increases in all three treatments. The enzyme activity of AP increased in treatment “UVW” (+29.1 %) and LAP in treatment “Herbicide” (+25.4 %) the most.

Table 17: Comparison and increase of enzyme activity (EA) of AP and LAP in standard and mulch layer treatments

<b>comparison and increase in % of enzyme activity</b>						
<b>treatment</b>	<b>EA of AP standard in nmol/(g soil*hour)</b>	<b>EA of AP with mulch in nmol/ (g soil*hour)</b>	<b>increase in %</b>	<b>EA of LAP standard nmol/ (g soil*hour)</b>	<b>EA of LAP with mulch in nmol/ (g soil*hour)</b>	<b>increase in %</b>
Greening	135	163	20.8	206	243	17.7
Herbicide	110	129	17.9	160	201	25.4
UVW	102	132	29.1	170	202	18.9

Figure 28 show the differences between the standard under-trellis treatments “Greening”, “UVW” and “Herbicide”, with and without grass mulch layer. Enzyme activity of acid phosphates (Figure 23),  $\beta$ -glucosidase (Figure 25) and leucine aminopeptidase (Figure 27) increases under a grass mulch layer. The exception to this

statement is the enzyme activity of  $\beta$ -glucosidase and leucine aminopeptidase in the treatment “Greening” on July 30<sup>th</sup>, there is no increase in enzyme activity measurable.

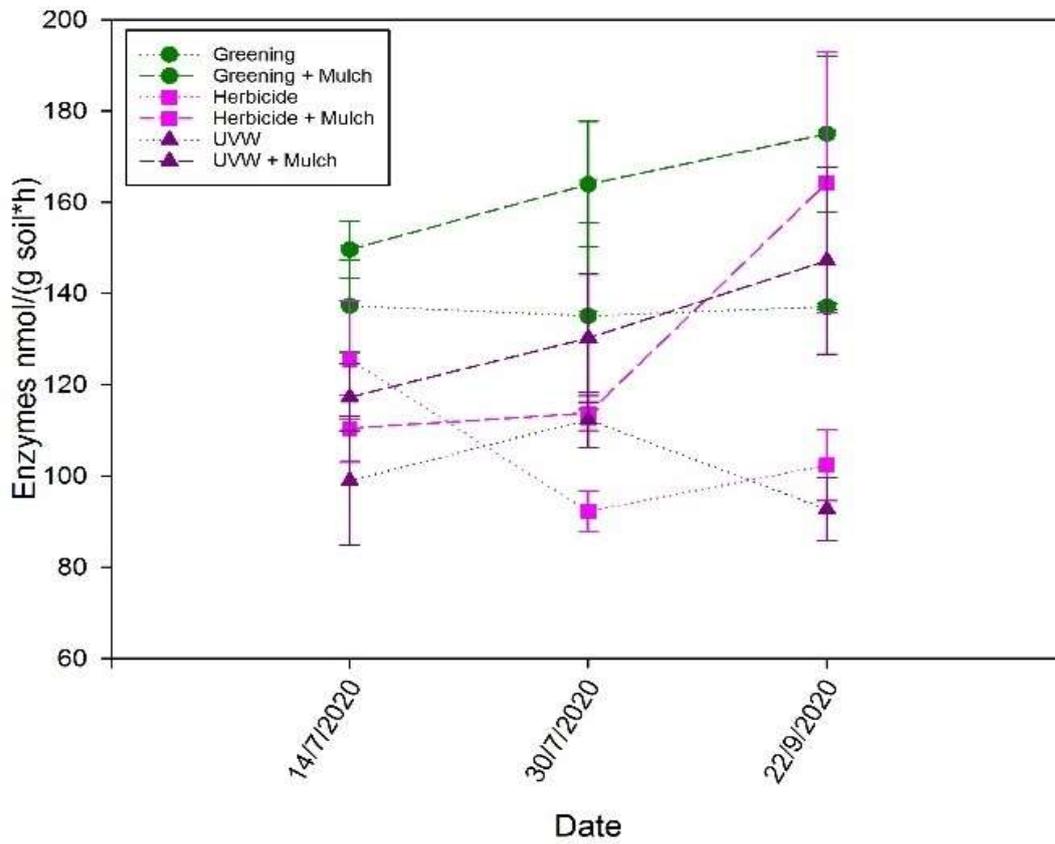


Figure 28: Comparison of the activity of acid phosphates under a grass mulch layer and in standard treatments

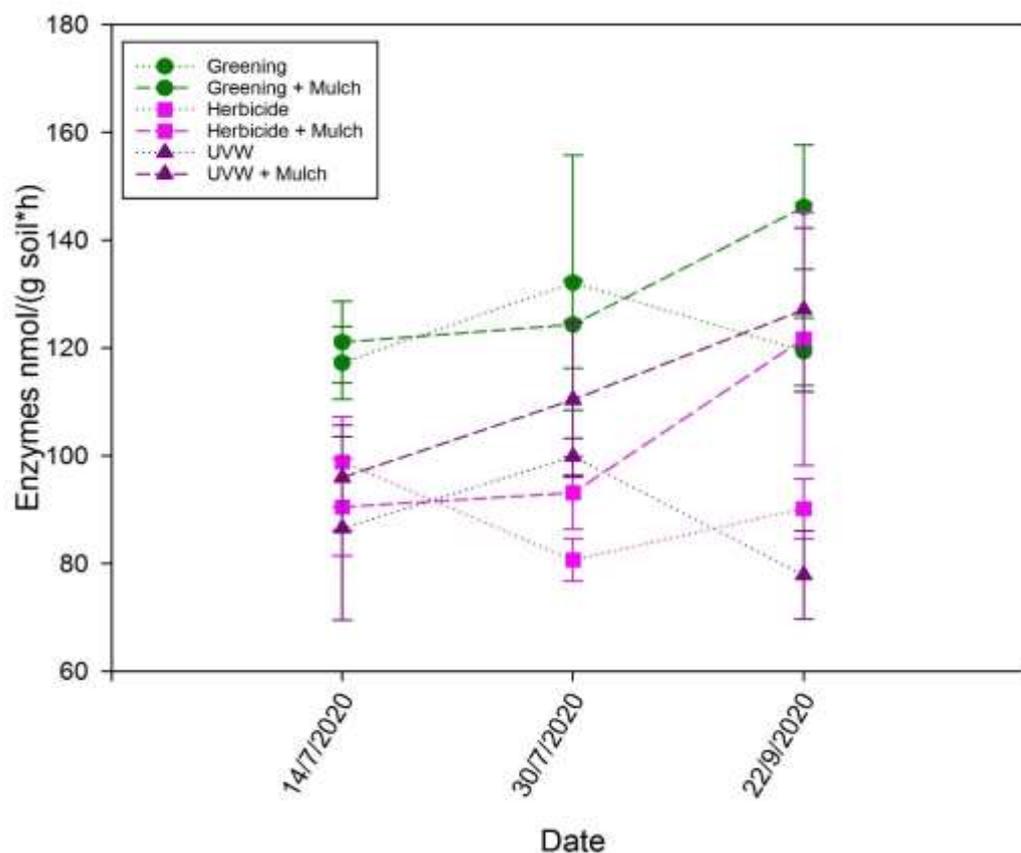


Figure 29: Comparison of the activity of leucine aminopeptidase under a grass mulch layer and in standard treatments

The values of the t-test in Table 18 show a high significant difference in the activity rates of all three enzymes between UVW treatments with or without grass mulch layer (p-value of AP: 0.009\*\*, of BG: 0.032\* and of LAP: <0.001\*\*\*). The grass mulch layer on the herbicide treatment only shows a significant effect on leucine amino peptidase (p-value:0.008\*\*). Furthermore, there is a significant effect of grass mulch layer on the enzyme activity of AP (p-value: 0.017\*\*) an LAP (p-value: 0.018\*) in the “Greening” treatment compared to “Greening+mulch”.

Table 18: P-values of t-tests of standard under-trellis strategies compared to mulch layer treatments concerning enzyme activity of acid phosphatase (AP),  $\beta$ -glucosidase (BG) and leucine aminopeptidase (LAP)

comparing p-values of t-test (standard vs. mulch layer treatment)			
under-trellis treatments	p-value (AP)	p-value (BG)	p-value (LAP)
herbicide+mulch - herbicide	0.104	0.280	0.008**
UVW+mulch – UVW	0.009**	0.032*	<0.001***
greening+mulch – greening	0.017**	0.450	0.018*

### 5.3 Fruit quality parameters

Figure 30 displays tartaric acid (a), pH (b), soluble solids (c) and NOPA (d) on September 3<sup>rd</sup> and 17<sup>th</sup>, shortly before harvest. In treatment “Control” the concentration of tartaric acid mostly decreased by 46.16 %. On 1<sup>st</sup> sampling date the treatment “Herbicide” shows the lowest value of tartaric acid (17.08 g/l) and only decreased by 37.65 %. The grape must of the treatment “Control” contains 1.0816 g/l soluble solids. The treatment “UVW” (1.0793 g/l) and “Herbicide” (1.0807 g/l) have less soluble solids than the other treatments.

Table 19: Values of grape most in standard treatments (tartaric acid, pH, NOPA and soluble solids) on 1<sup>st</sup> (September 3<sup>rd</sup>) and 2<sup>nd</sup> (September 17<sup>th</sup>) sampling date

Values of grape most								
under-trellis treatments	tartaric acid in g/l		pH		soluble solids in g/l		NOPA in mg/l	
sampling	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>
Greening	18.55	10.35	2.95	3.15	1.0579	1.0815	41.00	72.50
Rotary tillage	18.25	10.35	2.94	3.16	1.0589	1.0811	47.75	73.25
Herbicide	17.08	10.65	2.97	3.17	1.0595	1.0807	65.25	91.00
Control	18.63	10.03	2.93	3.17	1.0582	1.0816	48.50	70.25
UVW	18.23	10.10	2.97	3.15	1.0588	1.0793	47.75	72.50

The must yeast usable nitrogen (NOPA) is present in the treatment “Herbicide” (1<sup>st</sup>: 65.25 mg/l and 2<sup>nd</sup>: 91 mg/l), followed by “Rotary tillage” (47.75 mg/l and 73.25 mg/l), “UVW” (47.75 mg/l and 72.5 mg/l), “Greening” (41 mg/l and 72.5 mg/l) and finally the treatment “Control” (48.5 mg/l and 70.25 mg/l). Table 20 shows the developments of sugar content from September 3<sup>rd</sup> until September 22<sup>nd</sup>. The treatments “Greening” (77.50 Oe) and “Control” (77.13 Oe) reach the highest sugar contents.

Table 20: Sugar content of grapes in Oechsle (°Oe) on 1<sup>st</sup> (September 3<sup>rd</sup>) and 2<sup>nd</sup> (September 22<sup>nd</sup>) sampling date

Sugar content of grapes in Oechsle (°Oe)		
under-trellis treatment	1 <sup>st</sup> (September 3 <sup>rd</sup> )	2 <sup>nd</sup> (September 22 <sup>nd</sup> )
Greening	53.50	77.50
Rotary tillage	53.00	76.88
Herbicide	55.25	76.25
Control	54.25	77.13
UVW	54.25	75.63

Table 21 summarizes the results of the performed two-way ANOVA to test differences between tillage strategies, date, and interaction between tillage and date. The date appears as a high significant factor for all parameters ( $p$ -value:  $<0.001^{***}$ ). No significant differences on tillage variants are present concerning pH, tartaric acid, and soluble solids. Only the yeast-available nitrogen (NOPA) shows a significant dependence ( $p$ -value: 0.024) in conjunction with the under-trellis strategies.

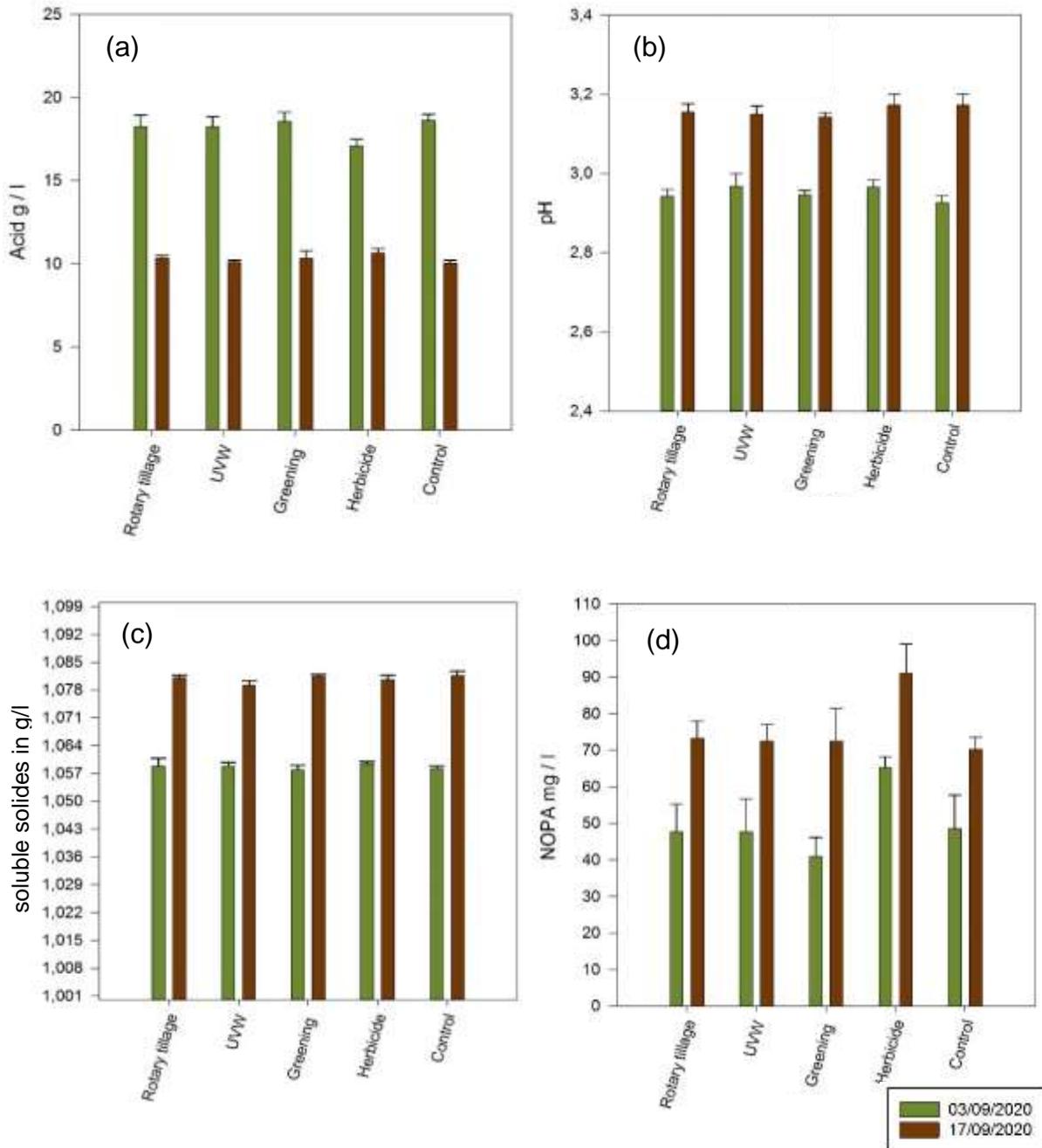


Figure 30: (a) Tartaric acid, (b) pH, (c) soluble solids and (d) NOPA of different under-trellis treatments subjected to date; brown: 1<sup>st</sup> sampling date (September 3<sup>rd</sup>) and green: 2<sup>nd</sup> sampling date (September 17<sup>th</sup>)

Table 21: P-value of ANOVA for influence of tillage and date on substance of content in grapes

	p-value			
	tartaric acid	pH	soluble solids	NOPA
tillage	0.699	0.857	0.869	0.024
date	<0.001***	<0.001***	<0.001***	<0.001***
tillage*date	0.132	0.647	0.514	0.966

Figure 31 and Table 22 show differences in berry and stock weight comparing the under-trellis management strategies. Berry weight (p-value: 0.334) and stock weight (p-value:0.841) are not significantly influenced by the treatments. Weight in g per grape does not differ significantly between the different treatments (greening: 140 g, rotary tillage: 140 g, herbicide: 148 g, control: 151 g, UVW: 143 g).

Table 22: P-value of berry and stock weight in dependence of tillage on both sampling dates

	p-value	
	berry weight	stock weight (weight in g/grape)
tillage	0.334	0.841

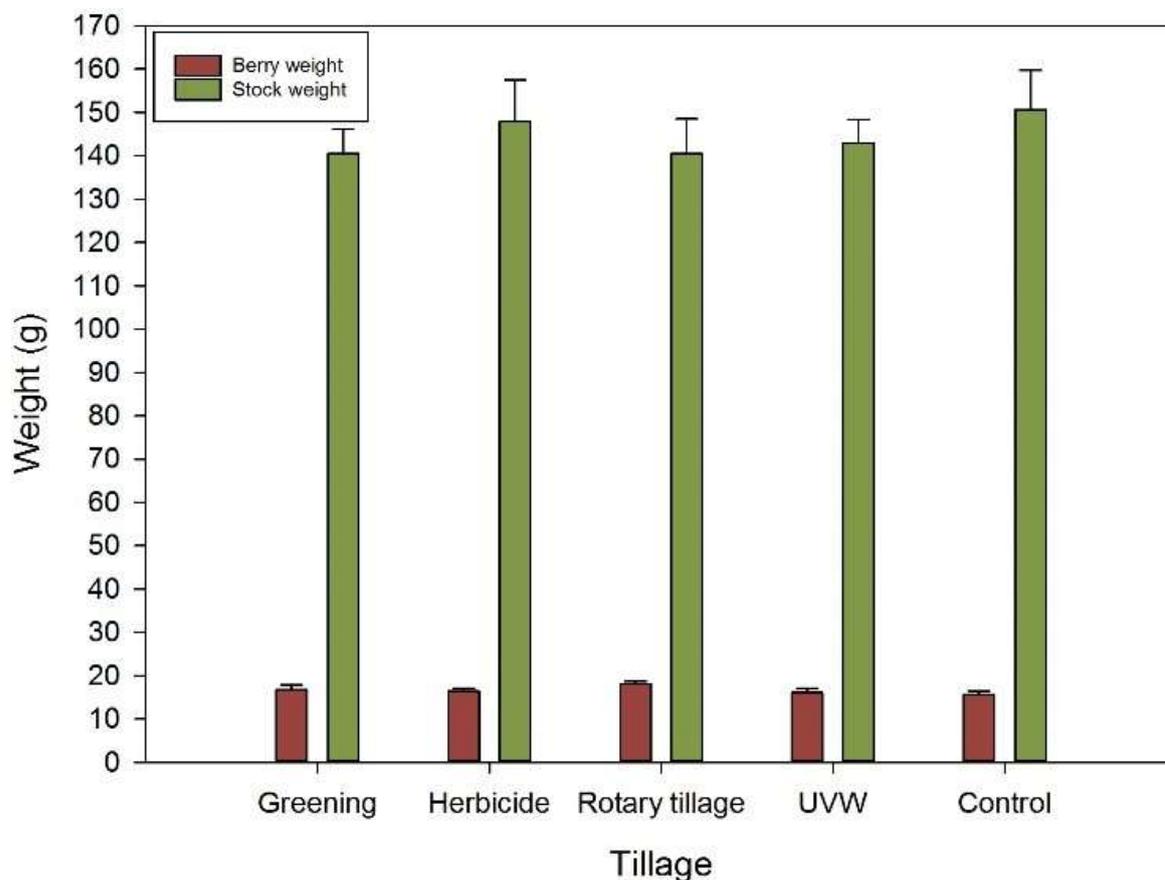


Figure 31: Influence of tillage strategy (Herbicide, Greening, Rotary tillage, UVW and Control) on berry weight and stock weight, brown: 1<sup>st</sup> sampling date (September 3<sup>rd</sup>) and green: 2<sup>nd</sup> sampling date (September 17<sup>th</sup>)

## 6 Discussion

The aim of the study was to investigate the influence of different under-trellis management approaches on soil and vine vigour. In order to achieve this aim, analyses of the soil moisture, the enzyme activity, the yield and the fruit quality were conducted. A healthy soil is one of the most important factors for balanced vine growth and wines of excellent quality and taste. An even supply of water and nutrients to grape vines ensure a sustainable yield and quality. Management strategies of a vineyard have to be planned in detail (Bauer et al., 2017) and balance vegetative and reproductive growth (Kliewer and Dokoozlian, 2005). Influencing factors and the present status of soil moisture, enzyme activity and ripeness parameters of different under-trellis treatments represent soil quality and enable interpretations referring vine vigour.

According to the results of this master`s thesis, soil moisture at 0-10 cm and 10-20 cm is significant influenced by precipitation in treatment “UVW” and “Control” Under vine-wheelers perform intensive soil cultivation and ground cover is disturbed. Under-trellis management systems in general influence water and soil biodiversity of microbes (Ripoche et al., 2010) and decreases stress levels of nutrient and water deficiency (Schubert, 2006). Therefore it is gratifying, that the results of this master`s thesis show a significant influence of under-trellis strategy on soil moisture in 0-10 cm (p-value: 0.004\*\*) and 10-20 cm (p-value: <0.001\*\*). This current study points out, that treatment “Herbicide” has the most soil moisture in all investigated soil layers.

Hofmann et al., (2014) determined that low-growing cover crops and natural growing plants are recommendable to establish a vegetation cover, this statement is in accordance with the findings of this study. Rotary tillage shows the lowest soil moisture in all samplings. An explanation to this low soil moisture values is that rotary tillage leads to a loss of soil moisture by -11.23 % in 0-10 cm and -25.35 % in 10-20 cm.

The tillage treatment “UVW” results in approximately equal soil moisture loss in all soil layers when comparing all sampling dates. UVW is ranked 4<sup>th</sup>, when comparing soil moisture of 0-10 cm and ranked for 2<sup>nd</sup> most soil moisture in 10-20 cm. Steenwerth et al., (2016) observed that mechanical tillage differs from mown treatments. This statement confirms with the results of this study, because of a significant difference between “Rotary” and “Herbicide” tillage. Treatment “Greening” and “Control” show similar value of soil moisture in 10-20 cm and remarkable more than the treatment

“Rotary tillage”. In comparison, Celette et al. (2009) observed that soil moisture of cover cropped treatments is increased compared to mechanical tilled treatments. In no-till treatments soil moisture is maintained by a reduction of evaporation caused of cover crops or weeds (Steenwerth et al., 2016).

Hickey et al. (2016) stated that soil moisture at shallow soil layers is lower; this is consistent with the results of this study. Cover crops or weeds take water from shallow layers (Hickey et al., 2016), but vine can compensate water deficiency by root growth to deeper layers (Steenwerth et al., 2016). Therefore negative effects on vine vigour can be avoided. The treatment “Rotary tillage” is an exception, because of the lower soil moisture content in deeper layers. Moisture loss in deeper layers could be caused by the processing horizon of the rotary tiller and finger weeder. In 10-20 cm sampling depth the UVW treatment shows higher soil moisture than “Greening” and “Control”. Generally, it is important to stress, soil moisture of the different under-trellis strategies in 10-20 cm differ more, than in shallow layers (Kliewer and Dokoozlian, 2005). To discuss high levels of soil moisture in herbicide treatments, it has to be mentioned, that ground cover systems negatively influence vegetative growth by water uptake. Therefore higher plant density or intensive under-trellis cover crop systems reduce water potential in soils (Coniberti, Ferrari, Disegna, García Petillo, et al., 2018).

The implementation of a grass mulch layer significantly influences soil moisture of 0-10 cm in the treatments “Herbicide”, “UVW” and “Greening”. A grass mulch layer mostly increases the soil moisture of the treatment “Greening”. Soil moisture of “UVW” treatment has been increased at least. The grass mulch layer has higher impact on soil moisture at 0-10 cm depth in all three variants. According to Steenwerth and Guerra (2012), a grass mulch layer sustains soil moisture, the lack of light suppress germination and mulch layers facilitate root growth.

This study shows that enzyme activity is more influenced by tillage than date. Enzyme activity of AP, BG and LAP in the standard treatments “UVW” and “Herbicide” significantly differ from “Greening” and “Control” treatment. The highest enzyme activity of acid phosphatase,  $\beta$ -glucosidase and leucine aminopeptidase occurs in greening and control treatment. These results are confirmed by Peregrina et al., (2014) and Whitelaw-Weckert et al., (2007), which stated that permanent vegetation of under-

trellis area enrich biological activity of soils in vineyards. Furthermore the fungal richness of soils is enhanced (Hendgen et al., 2018) and the biological density of soil biota is important for regional wine typicity (Belda et al., 2017). The lowest enzyme activity occurs in the treatment "Herbicide". Briones and Schmidt, 2017 are convinced that soil biota is modified by plant biomass and soil quality. The intensity of tillage also influences soil biota (Briones and Schmidt, 2017). In addition to this, it can be mentioned that vegetation and plant diversity in a vineyard promote soil fertility and biodiversity (Buchholz et al., 2017; Garcia et al., 2018). Higher microbial activity rates improve soil quality according to SMAF (Mbutia et al., 2015). Complementary, under-trellis management by herbicide and under-vine weeder have the lowest enzyme activity and do not significantly differ from each other. The under-trellis management by UVW and the use of herbicide leads to a reduction in soil organisms, characterized by a decrease in enzyme activity of LAP, BG and AP. Bavougian and Read, 2018 base this result by the fact, that established permanent cover crops or natural vegetation permit the growing of resident vegetation and tender the habitat of soil organisms. Herbicides decrease mycorrhization of vine and colony-forming units in soils (Zaller et al., 2018). Greening and control (natural greening variant) treatment do not show a significant difference in enzyme activity. These results can be justified by the study of Amelung et al. (2018), as soil biota favours areas of nutrient availability, present of energy sources, areas of soil litter and plant roots. Greening and control treatments have higher amounts of plant roots and indirectly higher amounts of soil litter (Amelung et al., 2018).

Results of this master's thesis can be justified by the study of Bavougian and Read (2018), that show that differences between mulch and non-mulch layers occurs. After the implementation of a grass mulch layer enzyme activity of acid phosphatase and leucine aminopeptidase) significantly increases in all three treatments. AP in the treatment "UVW" and LAP in the treatment "Herbicide" have the lowest amount of enzyme activity in the standard variant. Both treatments (Herbicide +25,4%; UVW+29.1%) do the greatest increase, when applying a grass mulch layer.

Generally, a mulch layer enhance the number of omnivores and insects (Addison et al., 2013). Microbes favour moderate soil temperature and grass mulch raises soil temperature to an optimum compared to exposed soil surface (Amelung et al., 2018).

Higher rates of enzyme activity are a possible reason for temperature rise, because soil biota is more active and produces more energy. In comparison, cover crops decrease temperature caused by higher transpiration rates (Steenwerth and Belina, 2008). The mulch layer acts as a protective cover for soil surface (Merwin et al., 1994), reduces evaporation loss (Chan et al., 2010) and microbes are more active under a mulch layer caused by proper soil water content (Jacometti, 2007). The study shows that a low enzyme activity in the standard variant leads to a higher potential for enhancement of enzyme activity under a grass mulch layer. Greening treatment shows a higher enzyme activity of LAP, AP and BG under grass mulch layer compared to “UVW” and “Herbicide”.

Natural or seeded cover crops and the implementation of a mulch layer promote enzyme activity. The results are also confirmed with Buchholz et al. (2017), that microbes in soils are less affected by tillage than by plant biomass and soil quality. Vineyards with high soil quality exemplify high quantities of soil biota (Buchholz et al., 2017).

The master`s thesis analysis results do not show a significant effect of different tillage treatments on ripeness parameters like pH, tartaric acid and soluble solids. A competition for potassium between vine and cover crops could have a reducing impact on pH of berries (Mpelasoka et al., 2003). The pH of cover crop treatments “Greening” and “Control” was similar to mechanical tillage treatments. The initial situation of soil parameters at study site presents a loamy silt texture (Bundesforschungs- und Ausbildungszentrum für Wald, 2021). Loam indicates high soil quality in combination with a high water capacity triggered by silt fraction (Amelung et al., 2018). This well-supplied soil could be a reason for the absence of differences in must composition. Results suggest that the vineyard manager of the study vineyard implemented a balanced cultivation system. Winter et. al. (2018). are convinced that winegrowers have to handle long-term ecosystem services and also have to earn money. The combination of these two goals represents a difficult paradox. The study showed that soluble solids do not significant differ between the treatments. The treatment “Greening” shows the highest amount of sugar, and it is an under-trellis strategy, that protect nature.

In contrast, the results show an impact of under-trellis strategy on yeast-available nitrogen (NOPA). The most yeast usable nitrogen (NOPA) is present in the treatment with herbicide, UVW and rotary tillage. Hickey et al. (2016) complements this statement, because the under-trellis management affect nitrogen, potassium, magnesium, and phosphorus. Mechanical tillage leads to the release of nitrogen, this can be absorbed by the vine and stored in the grapes. Furthermore, the diminished increase in treatment "Control" of NOPA from September 3<sup>rd</sup> to 17<sup>th</sup> and the least amount at harvest indicates the necessity of a adapted under-trellis management system. Donnini et al., (2016) could not find results, which determine significant variations between mechanical and herbicide treatment in berry weight, total soluble solids, pH and tartaric acid. Consistent with the study of Donnini et al. (2016) the results of this master`s thesis clearly indicate that the tillage strategy is not influencing berry weight. As counter argument the results of this master`s thesis displays higher NOPA rates in herbicide treatment. Zaller et al. (2018) discovered different contents of magnesium in leaves and nitrogen in grape juice between mechanical and chemical treatments. The study shows that sugar content of grapes and NOPA are lightly affected by under-trellis management. Cover crops and mechanical tillage do not influence cluster number (Hickey et al., 2016) per vine nor yield (Pérez-Álvarez et al., 2015). Compared to plant density, Conibert et al. (2018) found a greater effect of under-trellis strategy on yield.

Finally, it is important to stress that treatment "Greening" and "Control" the most recommendable option, that have to be discussed in the next chapter.

## 7 Conclusion and perspective

Biodiversity loss and the decline of ecological degradation (Bavougian and Read, 2018) are tremendous issues winegrowers are confronted with (Tilman et al., 2002). Regulation and supporting systems have an impact on vineyards (Garcia et al., 2018). The choice of the appropriate under-trellis strategy has an impact on wine quality and the continuity of the vineyard more than before.

Under-trellis strategies and grass mulch layers influence soil moisture and enzyme activity in different values. Soil moisture strongly depends on precipitation rates and in 0-10 cm the mechanical tillage influences soil moisture negatively. The priority objective in future is the improvement of soil quality. Therefore, cover crops or natural vegetation should be implemented to enhance enzyme activity and rise soil biota. The usage of a rotary weeder leads to a loss of soil moisture, and it is not desirable. Furthermore, an under-vine weeder should be used with caution in 0-10 cm procession horizon to prevent unnecessary loss of soil moisture. Concerning soil moisture, the cultivation by an under-vine weeder in 10-20 cm is a selectable option, but the usage of a UVW reduces enzyme activity. Under-trellis management strategy by herbicide due to high soil moisture and low enzyme activity rates. There for the usage of herbicide in the under-trellis area is not recommendable for vineyards. Complementary, it is important to point out, that under-trellis management strategies indirectly impact vine vigour in a complex system of different determinants like soil moisture and enzyme activity. Higher rates of enzyme activity are recognizable in cover cropped treatments.

Undoubtedly, a grass mulch layer increases soil moisture and enzyme activity (AP and LAP), primarily at 0-10 cm depth. The implementation of a grass mulch layer represents a good complement, if the costs are reasonable. In the last decade, glyphosate has been the most used herbicide for weed management (Siehl, 1997), replaced by mechanical tillage in viticulture (Merwin et al., 1994). In the future, a change in thinking will have to take place. The implementation of seeded or natural ground cover, a reduced intervention in nature and working with nature represent the only true solution. The water saving effects of no-tilled systems indicate the need to incorporate ground covered under-trellis strategies.

The results show that the use of herbicides has a negative effect on soil enzymes, and it is not recommended. Actually, impacts of soil management strategies on soil and wine quality need long-term studies (Al-Kaisi et al., 2005). The decomposition and nutrient cycle of enzymes should be examined (Steinweg and McMahon, 2012). A recommendable perspective for further studies is the planning of a long-term study to investigate the effects of ground cover and mulch layers at the under-trellis area to verify the activity of soil organisms and their composition. Further studies on proper under-trellis management strategies of cropped treatments and plant diversity are desirable.

Quantity of soil bacteria and fungal composition are significantly increased under organic managed soils (Hendgen et al., 2018). Winegrowers should aspire balanced ecosystems, optimized plant cultivation (Hofmann et al., 2014) and wine quality. Working with nature to reach a balance in vineyards and personal life is desirable.

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## 11 Appendix

Table 23: Scheme for pipetting: Plate set-up standard curve – 50  $\mu$ M dilution

	1	2	3	4	5	6	7	8	9	10	11	12	
A	0	0	5	5	10	10	15	15	20	20	25	25	blank (buffer)
B	0	0	5	5	10	10	15	15	20	20	25	25	sample 1
C	0	0	5	5	10	10	15	15	20	20	25	25	sample 2
D	0	0	5	5	10	10	15	15	20	20	25	25	sample 3
E	0	0	5	5	10	10	15	15	20	20	25	25	sample 4
F	0	0	5	5	10	10	15	15	20	20	25	25	sample 5
G	0	0	5	5	10	10	15	15	20	20	25	25	sample 6
H	0	0	5	5	10	10	15	15	20	20	25	25	sample 7

Table 24: Scheme for pipetting: 200  $\mu$ L buffer/soil slurry addition to standard curve plate

	1	2	3	4	5	6	7	8	9	10	11	12	
A	200	200	200	200	200	200	200	200	200	200	200	200	blank (buffer)
B	200	200	200	200	200	200	200	200	200	200	200	200	sample 1
C	200	200	200	200	200	200	200	200	200	200	200	200	sample 2
D	200	200	200	200	200	200	200	200	200	200	200	200	sample 3
E	200	200	200	200	200	200	200	200	200	200	200	200	sample 4
F	200	200	200	200	200	200	200	200	200	200	200	200	sample 5
G	200	200	200	200	200	200	200	200	200	200	200	200	sample 6
H	200	200	200	200	200	200	200	200	200	200	200	200	sample 7

Table 25: Scheme for pipetting: Buffer addition to assay plate

	blank		sample 1		sample 2		sample 3		sample 4		sample 5	
	1	2	3	4	5	6	7	8	9	10	11	12
A	250	200	50		50		50		50		50	
B	250	200	50		50		50		50		50	
C	250	200	50		50		50		50		50	
D	250	200	50		50		50		50		50	
E	50		50		50		50		50		50	
F	50		50		50		50		50		50	
G	50		50		50		50		50		50	
H	50		50		50		50		50		50	
	sample 6		sample 7		sample 8		sample 9		sample 10		sample 11	

Table 26: Scheme for pipetting: Soil sample addition to assay plate

	blank		sample 1		sample 2		sample 3		sample 4		sample 5	
	1	2	3	4	5	6	7	8	9	10	11	12
A			200	200	200	200	200	200	200	200	200	200
B			200	200	200	200	200	200	200	200	200	200
C			200	200	200	200	200	200	200	200	200	200
D			200	200	200	200	200	200	200	200	200	200
E	200	200	200	200	200	200	200	200	200	200	200	200
F	200	200	200	200	200	200	200	200	200	200	200	200
G	200	200	200	200	200	200	200	200	200	200	200	200
H	200	200	200	200	200	200	200	200	200	200	200	200
	sample 6		sample 7		sample 8		sample 9		sample 10		sample 11	

s

Table 27: Scheme for pipetting: Enzyme substrate addition to assay plate

	blank		sample 1		sample 2		sample 3		sample 4		sample 5	
	1	2	3	4	5	6	7	8	9	10	11	12
A		50		50		50		50		50		50
B		50		50		50		50		50		50
C		50		50		50		50		50		50
D		50		50		50		50		50		50
E		50		50		50		50		50		50
F		50		50		50		50		50		50
G		50		50		50		50		50		50
H		50		50		50		50		50		50
	sample 6		sample 7		sample 8		sample 9		sample 10		sample 11	