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Influence of `terroir` factors and vineyard management (organic, biodynamic) on plant performance and fruit quality of grapevines

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Ort, Datum

Johanna Döring

Contents

| | | |
|-----------|---|-----|
| Chapter 1 | General Introduction..... | 1 |
| Chapter 2 | Indirect Estimation of Leaf Area Index in VSP-Trained Grapevines Using Plant Area Index ¹ | 32 |
| Chapter 3 | Soil water-holding capacity mediates hydraulic and hormonal signals of near-isohydric and near-anisohydric <i>Vitis</i> cultivars in potted grapevines ² | 39 |
| Chapter 4 | Growth, Yield and Fruit Quality of Grapevines under Organic and Biodynamic Management ³ | 50 |
| Chapter 5 | Organic and Biodynamic Viticulture affect Soil, Biodiversity, Vine and Wine Properties: a Systematic Quantitative Review ⁴ | 79 |
| Chapter 6 | General Discussion..... | 105 |
| Chapter 7 | Summary..... | 130 |
| Kapitel 8 | Zusammenfassung | 134 |

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³Döring J., Frisch M., Tittmann S., Stoll M., Kauer R. 2015. Growth, Yield and Fruit Quality of Grapevines under Organic and Biodynamic Management. PLOS ONE **10**(10): e0138445. <https://doi.org/10.1371/journal.pone.0138445>.

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Chapter 1 General Introduction

Grapes and wines are highly differentiated and complex agricultural products. Their quality is determined by the interaction of a wide range of factors such as variety, vintage, soil, vineyard management, and winemaking practices and is difficult to define [102]. Determination of wine quality must ultimately be done by subjective factors such as taste, sight, and smell. Linking chemical analysis to sensory evaluation of taste panels would be ideal for determining quality, but given the chemical complexity of wine this is still difficult [44,79]. The most important chemical quality variables are sugar concentration, pH and titratable acidity in the berry at harvest, phenolics, anthocyanins, different aroma compounds and the absence of contaminants derived from pests and diseases. These quality parameters can potentially be influenced by different viticultural, environmental as well as enological factors (Fig. 1) [44,102].

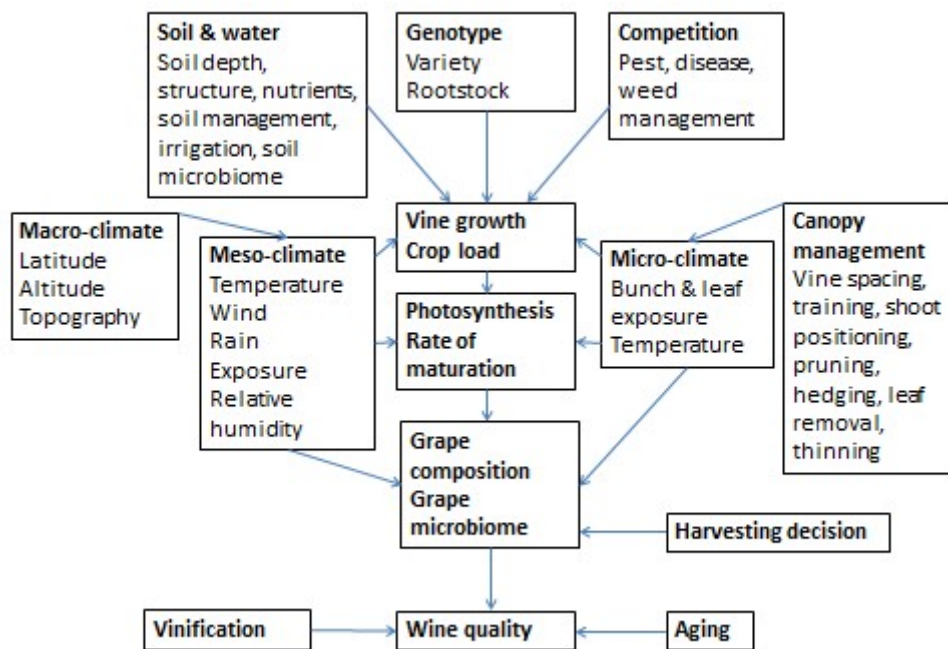


Fig. 1: Environmental and viticultural inputs into grape composition and wine quality (modified according to [44]).

Traditionally the awareness for quality is much more pronounced among winegrowers than it is among farmers of annual crops and delimitations of origin in viticulture have a millennia-old tradition [91]. Since the classical age written documents exist that prove the fame of several specific regions for winegrowing [9,15]. In middle-age France monks in Burgundy established an elaborated system to rate vineyard sites according to

their suitability to grow high-quality wines [36]. In the 20th century delimitations of origin have been introduced in most of the winegrowing countries worldwide [91]. Grapevines might be the crop where the concept of quality is linked to 'terroir' the most. The concept of 'terroir' means that a wine produced under certain conditions in a specific region is unique and cannot be reproduced [2]. The existence and definition of the terroir concept is one of the most debated issues in oenology and viticulture [19,86]. One reason for this might be that the concept of terroir includes not only a scientific, but also a cultural aspect [91].

Fruit quality is highly linked to plant performance, since optimum plant performance is the pre-requisite for producing high-quality crops [44]. The perception of optimum plant performance – in line with the perception of quality – substantially differs between agriculture and viticulture, as well. The awareness among winegrowers that optimum plant growth is not necessarily maximum plant growth is also distinct and has a long tradition in viticulture.

A lot of research has been dedicated to the link between plant performance (vine growth, crop load and photosynthesis) and fruit quality in viticulture since decades. It is clear that optimum plant performance and growth are highly linked to viticultural as well as environmental conditions and cannot be generalized. Still some measures have been established that describe plant performance in an adequate way, such as leaf area to fruit weight-ratio [46].

'Terroir' factors determining plant performance and fruit quality in viticulture

Plant performance and fruit quality are highly influenced by the physical environment in which the agricultural crops grow [92]. The geology, geomorphology, the topography of the site, meaning the height, the slope and the exposition, the macro- and microclimate as well as soil traits in the different layers all determine plant performance and fruit quality of agricultural crops [40,92]. In viticulture all these factors are often summarized and described as 'terroir' referring to the specific designation of origin of a wine [40,92] and the factors interact with each other. In viticulture the concept of 'terroir' has long been acknowledged as an important factor determining wine quality and style [92]. Especially in France this concept was applied in the Bordelais region in the second half of the 19th century and by the introduction of the AOC system (Appellation d'Origine Contrôlée) in the first half of the 20th century it gained more importance [40]. Lately the existence of a terroir-specific effect on the transcriptome and metabolome of grape berries could be revealed [2]. The Organisation Internationale de la Vigne et du Vin (OIV) refers to the term 'terroir' as follows: "A terroir is a unique and delimited geographic area for which there is a collective knowledge of the

interaction between the physical and biological environment and applied viticultural practices. The interaction provides unique characteristics and creates recognition for goods originating from that area. Terroir includes specific characteristics of soil, topography, climate, landscape and biodiversity. “ [8]. Deloire *et al.* [18] define terroir as “a spatial and temporal entity, which is characterized by homogeneous or dominant features that are of significance for grapes and/or wine; i.e. soil, landscape and climate, at a given scale-duration, within a territory that has been founded on social and historical experience and genotype related technical choices”. According to Seguin [80] a good terroir should have the following properties: (1) adequate, but not excessive fertility, especially with respect to nitrogen; (2) ability to ameliorate the effect of heavy rain; and (3) ability to survive drought in very dry years. The geology determines soil traits, the soil traits partially have implications for the microclimate, but the climate also determines weathering of the rock of origin, water content of the soil and soil temperature. On the other hand the topography influences soil characteristics as well as microclimatic conditions of a specific site [40].

However, agricultural practices always influence the natural environmental factors summarized as ‘terroir’. Soil management influences soil traits such as water-holding capacity, soil aeration and soil temperature and fertilization strategies determine the nutrient supply of the plants and therefore actively contribute to the composition of different ingredients. These factors might also influence reactions of the plants to different pests or fungal and bacterial pathogens. Viticultural practices such as terracing introduce changes in the topography of the agricultural site and therefore also influences the ‘terroir’. All these mentioned changes of the environmental factors introduced by agricultural practices imply new interactions among the different ‘terroir’ factors and make the whole concept of ‘terroir’ even more complex. Furthermore, the different management steps such as pruning, the choice of the vineyard site, the variety and the rootstock, the trellis system, the canopy management, yield reduction, pest management and the timing of all the management steps determine the final wine quality and are part of a wider concept of ‘terroir’ [40]. Hoppmann estimated the physical environmental factors to contribute to the content of total soluble solids in wines from the Rheingau region by 70 %, whereas 30 % were influenced by the single winegrower through soil management, fertilization, yield reduction, trellis system, canopy management including defoliation and pest management strategy [39]. It is estimated that the share of the contribution of the management influenced by the winegrower is similar concerning the total acidity of the harvested grapes, but it might be even higher concerning other ingredients, since management steps such as canopy management or trellis system might influence secondary metabolites in the berries even more [40]. By selecting the vineyard site itself, the variety, the clone and the rootstock the winegrower influences the taste of the wine to an even higher extend. All these factors are often called the ‘human factor in terroir’ [92].

Climate with its various aspects highly determines plant performance and fruit quality not only in viticulture depending on the tolerance and the needs of the single agricultural crop species. Temperatures, rainfall, vapor pressure deficit (VPD), evapotranspiration, solar radiation and wind are important aspects of the climatic conditions that influence vine phenology and performance [92]. By means of agro-climatic indices the influence of these factors delimiting vine growth and grape ripening can be described [43,98]. The yearly variation of the climatic conditions in viticulture highly determines final wine quality and is known as the 'vintage effect'. Climatic conditions are also highly dependent on the geographical coordinates of latitude and longitude. Within these macroclimates topoclimatic, landscape related conditions (mesoclimate) largely influence vine performance and winegrape quality. Microclimatic variability at plot level or inside the canopy highly determines berry quality traits [92].

Air temperature is a very important factor delimiting vine growth. Therefore air temperature plays a major role in determining the spread of viticultural areas throughout the world. However, air temperature is also highly influenced by geomorphology and topography [92]. The length of growing seasons highly influences vine performance and final wine quality. Especially minimum winter temperatures delimit vine growth worldwide [40]. Besides that photosynthesis is highly temperature-dependent and is maximized at 25 °C [1]. Accumulation of metabolites in the berry is also temperature-related. Grape sugar concentration is maximized between 25 and 30 °C and anthocyanin accumulation is maximized between 17 and 26 °C [40,44]. Acidity and pH in the berry are also temperature-dependent. The degree of malic acid reduction is related to temperatures after veraison. Higher temperatures before veraison, in contrast, are associated with greater malic acid accumulation in the berry. Some studies indicate that cool climates produce more flavor and aroma constituents. Still the temperature effect is difficult to separate from the effect of light income [44].

The amount of rainfall is crucial for plant development and crop quality. Average rainfall among winegrowing regions varies between 300 and 1000 mm per year, and most quality wines are produced in winegrowing areas with precipitation lower than 700 to 800 mm per year [44,92]. A lot of important winegrowing regions worldwide are located in dry climates and are reliant upon supplemental irrigation. Vine-water status does not exclusively depend on the amount of rainfall or irrigation water supply, but evapotranspiration and soil water-holding capacity also determine the water status of the plants. Rainfall does not only ensure water supply of agricultural crops, but may also contribute to an increase in disease pressure [92]. This is why heavy rainfall and excessive irrigation lower quality [44]. In viticulture downy mildew (*Plasmopara viticola*) and Botrytis bunch rot (*Botrytis cinerea*) together with berry splitting are particularly favored by high rainfall events throughout the growing season [44,92]. Furthermore, heavy rainfall during maturation of the berries might induce lower ripening, higher yields, higher acidity and lower anthocyanin content. Inversely, water

stress does not always have positive implications for the ripening process. The amount of water stress and its timing are important for determining berry and wine quality [44].

Photosynthesis is closely linked to solar radiation and increases with solar irradiance [92]. The optimum photosynthetically active radiation (PAR) for photosynthesis is $700 \mu\text{mol m}^{-2} \text{s}^{-1}$. More solar radiation will result in higher yields and/or higher total soluble solids [44]. But the effects of solar radiation and temperature are difficult to separate from each other [92]. Many secondary metabolites such as phenols and aroma compounds in berries are sensitive to solar radiation and temperature. The spatial variability concerning solar radiation and temperature within one plot or within a canopy can be very high and therefore variability of berry or grape quality within one plot or canopy was shown to also be high [28,29].

The effect of geology on wine quality and terroir expression is very controversially discussed [42]. One hypothesis is that minerals in the soil from weathering parent material might provide the vines with certain metabolites or ions responsible for a distinct sensory characteristic in the final wine. Others speculate that geology might influence wine typicity rather than wine quality. Still others think geology indirectly determines wine quality by determining soil type, soil depth, drainage, soil color and texture, soil mineral composition as well as soil water-holding capacity [42,92]. It is sure that geology determines and interacts with a lot of other natural environmental factors mentioned above and plays a decisive role in determining vine performance and fruit and wine quality. Geomorphology is closely linked to the geological origin of every landscape and indirectly influences wine quality by determining soil depth, soil mineral composition, share of rock in the soil, altitude, slope and exposure [92].

Soil in its complexity highly determines plant performance, fruit quality, wine quality as well as wine style. Still worldwide wine production occurs on a wide range of different soils. Soil texture (pebble, sand, silt and clay content) is one important soil characteristic. It indirectly determines soil water-holding capacity, cation exchange capacity, root penetration and growth, temperature in the root zone due to its heat-retaining and light-reflecting capacity and water-draining or water-retaining properties of soils [44,92]. Soil mineral composition is another important soil feature that clearly influences vine growth and wine quality and a certain amount of nutritive elements is a prerequisite for plant growth [92]. Yet it is not clear to which extend wine quality and the content of any nutritive element are directly correlated [80]. Nitrogen is the nutritive element which mostly impacts plant performance and wine quality. Nitrogen availability is dependent on the soil type. High microbiological activity leads to high organic matter turnover and thus to mineralization of organic nitrogen [44,92]. High nitrogen levels lead to high vigor, high leaf area, high pruning weights, increases yield, berry size, juice acidity and decreases tannin and anthocyanin concentration [44,92]. Nitrogen concentration in the juice and juice sugar concentration are negatively correlated [12]. This is why excessive nitrogen availability is by tendency attributed to

poor red wine quality. Low vine nitrogen availability, in contrast, is tendentially not favorable for white wine production, since it potentially decreases aroma precursor concentration and increases tannin concentration in the berry, as mentioned above. This is unwanted in white wines. Though excessive nitrogen supply leads to excessive vigor, leaf area and Botrytis bunch rot [44,92]. Vine nitrogen availability is also highly dependent on vine water status and increases with soil water availability [92]. Soil potassium content is closely linked to the geological origin of the parent material. But there is no clear evidence about the effect of potassium for winegrape quality. High potassium content in the juice by tendency reduces acidity and increases pH [44,92]. Several of the most prestigious winegrowing regions worldwide are located in areas with high soil calcium content, for example Burgundy and Champagne. Calcium content might have an indirect effect on wine quality by increasing water drainage, root penetration into the soil and soil temperature. It indirectly limits soil nitrogen availability, as well [92].

Soil color potentially modifies temperature and spectral composition of reflected radiation. It is still unclear how these two factors influence canopy microclimate. On the other hand the effects of soil color and soil temperature are difficult to separate from each other, because the share of sunlight reflected from the soil depends on its color. The amplitude of soil temperature between day and night might potentially be much higher than the amplitude of air temperature. Soil temperature does not only depend on soil color, but also on water content of the soil and thermal conductivity of soil particles. Dry soils warm up faster than wet soils, whereas light colored soils reflect more sunlight in comparison to dark colored soils. These effects have different implications for grapevine growth and grape quality. High temperatures in the root zone induce precocity, soluble solids accumulation and decrease malic acid content in berries. On the other hand canopy microclimate is also influenced by the proportion of sunlight reflected from the soil and its thermal conductivity. In the morning air temperature above the soil is higher on a soil with a fine texture, whereas in the afternoon air temperature above a stony soil might be higher [92].

Soil biological activity is a highly specific trait of every site or field used agriculturally. By highly determining soil fertility the soil micro- and macrobiome plays a decisive role in ensuring plant growth, yield stability and fruit or crop quality in general by providing ecosystem services of which the relevance for agricultural production might not even be known to this day [92]. It is still unknown to which extend the soil microbiome impacts wine quality on one hand and to which extend it is modified by different vineyard management practices on the other hand [34,92]. Lately also the grape microbiome has been proposed to be part of the 'terroir' factors of a vineyard that determine wine quality. Undoubtedly the grape microbiome highly impacts final wine quality and is highly influenced by pedoclimatic conditions as well as by management practices [64]. An overview of how different management systems in viticulture affect the microbial community in the soil and on the grapes can be found in Chapter 4.

It is important to have a closer look on the hierarchy among the terroir factors: soil, climate and human factors in order to understand whether some factors are more important than others. By selecting the vineyard site itself, the variety, the clone and the rootstock the winegrower influences the taste of the wine to a high extend [92]. As mentioned above, Hoppmann estimated the contribution of physical environmental factors to the content of total soluble solids in wines from the Rheingau region to be about 70 % [39]. According to Hoppmann about 30 % were influenced by the vineyard management of the winegrower (soil management, fertilization, yield reduction, trellis system, canopy management including defoliation and pest management) [39]. Van Leeuwen *et al.* [93] did a detailed research on the importance of the natural environmental factors soil, climate and cultivar taking into consideration the performance of three different cultivars on three different soils during five different vintages. The climatic differences among the vintages were considered as the climatic factor. Variables concerning vigor, yield, berry composition, vine mineral status and vine water status were considered. Leaf area index and pruning weight were mostly influenced by the climatic conditions, but to a certain extend also by soil type and cultivar (Fig. 2) [93].

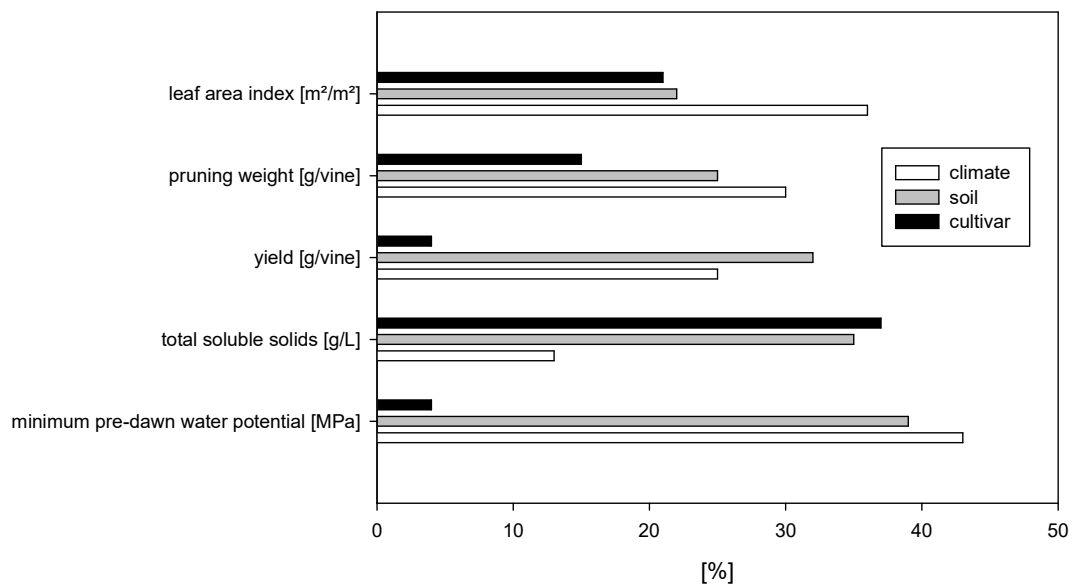


Fig. 2: Vigor, yield, total soluble solids and vine water status: Percentage of variance attributed to climate (vintage effect), soil and cultivar (modified according to [93]).

Yield showed to be equally determined by the climate and the soil factor (Fig. 2). Total soluble solids seemed to be mostly influenced by soil and cultivar and not by the climate (Fig. 2). Vine water status was highly determined by climate and soil, but not by the cultivar (Fig. 2). The results showed that the climatic factor had the strongest impact on the measured parameters, followed by soil type and cultivar. These main effects of

climate and soil type were mediated by vine water status, an important parameter in terroir expression. The human factor in terroir concerning vineyard management was not considered in this study [93].

Physiological response mechanisms of grapevines to water deficit

Vine water status plays a crucial role in terroir expression, since the effects of climate and soil type on plant performance and fruit quality in a specific vineyard site are equally mediated through vine water stress [94]. Vine water status depends on climatic conditions (rainfall, evapotranspiration), soil water-holding capacity as well as vineyard management (training system, plant material, irrigation etc.) [92]. Soil water-holding capacity is largely determined by pore size and depends on the soil type. Clay consists of very small pores ($<0.2\ \mu\text{m}$) that have the characteristic of holding water so firmly that plants are not able to extract it (water-retaining). Sandy soils, in contrast, consist of very large pores ($>10\ \mu\text{m}$ diameter) where water cannot be held against gravity and drains out of the soil (water-draining). Silt (pores between 0.2 and $10\ \mu\text{m}$ diameter) has the property to hold a lot of water which can be extracted by plant roots [4].

As mentioned before, vine water status is equally influenced by climate and soil properties [93,94] and mediates between these two important natural environmental terroir factors [92]. Its reliable assessment is crucial not only in terroir studies, because it has strong influence on plant performance and winegrape quality. There are three different approaches of determining plant water status: Soil water monitoring, water balance modelling or the use of physiological indicators. Pre-dawn leaf water potential and stem water potential are two of the most frequently used measures in physiological and terroir studies [92] and were also used in the studies in Chapter 2 and Chapter 3 to describe physiological responses of grapevines to environmental or management factors.

Pre-dawn leaf water potential as well as stem water potential are both measured by means of a pressure chamber [77]. Pre-dawn leaf water potential is usually measured shortly before sunrise, when stomata are closed and the plant reaches an equilibrium with the most humid layer of the soil. By detaching a leaf from the plant and pressurizing it in the pressure chamber until the water in leaf and petiole is forced to exude the tension the plant has to create to take up water from the soil can be determined. The more water stress there is, the greater the tension the plant has to build up in order to take up water from the soil and the greater the pressure required by the pressure chamber to cause water to exude [18]. Thresholds of pre-dawn leaf water potential have been proposed which offer the possibility to evaluate and compare the severity of the water deficit [7]. The development of the water status of the plant during the growing season in relation to the phenological stages of the vines provides good

information about the implications of vine water status for plant growth and berry ripening. Measures of pre-dawn water potential are highly dependent on the root profile of the plants, the soil depth and the texture and thus the variation of pre-dawn leaf water potential in one plot can potentially be high [18,92]. The advantage is that pre-dawn water potentials can give indication of plant water stress levels also in cool climates where cloud cover during the day regularly occurs within the growing season [88]. Stem water potential, in contrast, is measured during the day, usually at midday or in the afternoon when water stress levels reach a maximum [54]. Stem water potential is highly dependent on the transpiration rate. In order to obtain accurate measures leaves have to be bagged in aluminium foil one hour before measurement. By this the leaf whose transpiration is stopped reaches an equilibrium with the water potential in the stem [18]. Since solar radiation highly determines transpiration rate and thus stem water potential, stable solar radiation (PAR) during the day is one prerequisite for stem water potential measurements [95].

Grapevines are well-adapted to seasonal water deficit due to their deep and large root system and due to physiological drought avoidance mechanisms such as the efficient stomatal control of transpiration or the hydraulic control of transpiration via embolism formation and repair [11,52,53] or osmotic adjustment [73]. The severity of water stress determines how the physiological performance of the plant is influenced. Moderate water stress induces non-hydraulic stress signals (e.g. abscisic acid) which are sent from roots to shoots and which trigger stomatal closure and thus transpiration rate. In case of severe water stress embolism formation in xylem vessels occurs which leads to a decrease of hydraulic conductance. Moreover, permanent water stress may lead to modifications in xylem anatomy on a long term [54].

As mentioned before, grapevines cope with seasonal water deficits either by hormonal or by hydraulic regulation of transpiration and thus they control water consumption dependent on vine water status [11]. Stomatal closure is one of the early plant responses to mild or moderate water stress. It restricts water loss and carbon assimilation. Photosynthesis of grapevines is usually more resistant to drought compared to stomatal conductance. This means that intrinsic water use efficiency (A/g_s) is usually higher for plants under mild or moderate water stress compared to well-watered plants [11]. When water deficit persists acclimatization processes such as growth cessation and osmoregulation occur.

Drought-sensitivity greatly varies among cultivars. Genotypic differences in stomatal sensing of water deficits among cultivars are high. Especially the timing and the intensity of response mechanisms to water deficit are highly dependent on genotype due to variations in constitutive differences in leaf gas exchange, the plant's capacity to osmoregulate and plant hydraulics [11]. Xylem vessel sizes are highly dependent on grapevine variety. Larger vessels tend to be more susceptible to embolism formation under water deficit [13]. Stomatal control of transpiration prevents xylem embolism

formation. Higher vulnerability for cavitation might therefore go along with stronger stomatal control [78]. In general grapevines are considered to be a 'drought-avoiding species' with strong stomatal control. Still some varieties described as isohydric were shown to exhibit a more efficient stomatal control in response to drought than others described as anisohydric [11,78,81]. It was shown that varieties that exhibited an isohydric behavior with stronger stomatal control of transpiration under water deficit had higher concentrations of ABA in xylem sap compared to anisohydric varieties [81]. Still the same variety showed to behave differently concerning stomatal control depending on experimental conditions. On the other hand the delivery of hormonal drought signals such as ABA highly varies among rootstocks. It seems likely that the extend of stomatal control under water deficit varies in function of the rootstock, the climate (VPD and temperature) and intensity and duration of water deficit [11]. The relative importance of hydraulic and chemical signaling as plant responses to water deficits is still a matter of discussion [11]

Different methods for measuring whole-plant sap flow are available based on thermodynamic principles such as heat pulse velocity, trunk segment heat balance, stem heat balance, heat dissipation and heat field deformation [24]. The extent of embolism formation in petioles is determined by assessing the percent loss of conductivity (PLC) that is present in a petiole compared to its potential maximum conductivity [14,54,82]. Water flow through xylem vessels can be determined by flushing water through xylem portions such as petioles. This is usually done with a High Pressure Flow Meter (HPFM) [82]. For this purpose an initial low pressure P_i is applied and can be calculated for every plant species and variety depending on the diameter of xylem vessels [99]. Then a transient pressure increase is induced until a high pressure is reached in order to flush out all embolisms formed in the xylem vessels. This high pressure is maintained for two to three minutes. In the end the final conductivity of the xylem portion under low pressure P_i is determined and compared to the conductivity of the xylem portion under the same low initial pressure at the beginning of the measurement [54]. The difference between initial and final hydraulic conductivity of the xylem portion is a measure for the degree of embolism formation and is expressed as percent loss of conductivity PLC [53,54]. An exemplary measurement of hydraulic conductivity on a petiole of field-grown *Vitis vinifera* L. cv. Riesling on 08/19/12 at 4 pm local time in Geisenheim, Germany, shows a discrepancy between the initial and the final conductivity of 54 %, meaning that PLC is 54 % (Fig. 3).

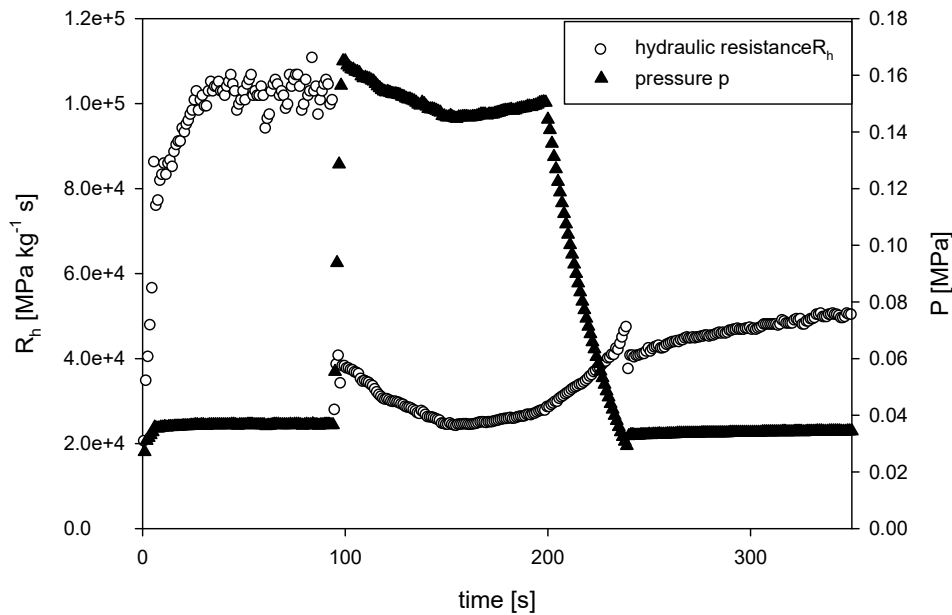


Fig. 3: Measurement of hydraulic resistance R_h on a petiole of field-grown *Vitis vinifera* L. cv. Riesling according to the pressure setup P in Geisenheim, Germany, on 08/19/12 at 4 pm (Döring unpublished).

Further details of the methodology of PLC assessment by HPFM measurements can be found in Chapter 3.

Implications of vine water status for plant performance and fruit quality

Vine water status has an effect on plant development and berry composition which is in many cases uncoupled from the cultivar effect [94]. Timing and intensity of water deficit influences berry development, metabolism as well as final composition, wine color and aroma [11,25,67]. The development of grape berries follows a double-sigmoid growth curve [16]. The two growth phases are separated by a lag phase. The beginning of the second growth phase is known as veraison [11]. Early water deficit stress usually occurs before veraison, whereas late water deficit stress occurs between veraison and harvest [94].

Early water deficit induces early shoot growth cessation and thus reduces vigor and leaf area [59,94]. Shoot length was reported to be around 25 % higher under well-watered conditions compared to an early season water deficit treatment. Moreover, shoot growth of vines under early season water stress stopped 50 days earlier compared to fully-irrigated vines. Compared to well-watered vines radial shoot growth ceased earlier

under early season water deficit, but differences in shoot diameter between treatments were less pronounced in comparison to radial shoot growth. It seems likely that low vine water status induces periderm development. Pruning weight was reduced about 10 % in early as well as late season water deficit treatments (*Vitis vinifera* L. cv. Cabernet franc). Photosynthetic rate is also reduced under water stress in grapevines and might mediate between plant water status and plant growth [59].

Yield differences between vines subjected to water stress and well-watered plants were observed by Etchebarne *et al.* [25], Matthews *et al.* [59] and Matthews and Anderson [58]. Vines under moderate to severe water stress showed significantly reduced yield per vine, berry weight as well as bunch weight. Berry fresh weight and water content were both significantly reduced compared to well-watered vines or even decreased under water deficit during ripening [25,33]. At early growth stages pre-veraison the berry is exclusively connected to the plant by the xylem. The influence of water deficit on berry growth is thought to occur directly through the amount of water transport through xylem vessels. On the other hand it seems possible that ABA synthesized as a reaction to water deficit limits cell division. As a consequence final berry size is reduced [11]. Post-veraison berries are connected to the vine via the phloem and impact of water deficits on berry size at this stage of ripening seem to follow more than one mechanism and might just occur indirectly due to a reduction of photosynthesis [11,25]. However, berry size and weight are mostly affected by plant water status between anthesis and veraison. Even if plants are consecutively well-watered between veraison and harvest, berry size decrease is often irreversible [33,58-60,67]. The growth phase that is most susceptible to water stress in grapevines seems to be the period from the beginning to four weeks after flowering [59,60]. Late season water deficit does not modify berry weight and diameter to the same extend as early water deficit does [33,60,67]. Fruit growth under late-season water deficit can be maintained in spite of low vine water status [59]. Reduction of the pericarp cell volume seems to be responsible for final berry size. The earlier the water deficit occurs, the more pericarp cell volume is reduced. It is likely that water deficit reduces cell wall extensibility and therefore berry size reduction due to early water stress is irreversible [67]. Phenological development of berry growth is also affected by wine water status. Early water deficits reduce the onset as well as the duration of the lag phase (*Vitis vinifera* L. cv. Syrah) [67]. Hardie and Considine, in contrast, found potted vines subjected to early season water deficit to enter later into veraison compared to well-watered plants (*Vitis vinifera* L. cv. Cabernet franc) [33]. Other studies did not observe differences in phenological development of grapevines under drought [58,59]. Seed weight, in contrast, does not seem to be influenced by vine water status [67]. Water stress in grapevines does not only influence yield, cluster weight and berry weight, but also makes an impact on fruitfulness of the subsequent year. Matthews and Anderson [58] observed yield decreases determined by early-season water deficits due to a decrease in cluster weight in the first experimental year. In the two following seasons they showed that early-

season water deficit influences fruitfulness and thus the number of clusters per vine as well as cluster weight of the subsequent year of the experiment. Again early-season water deficit effects were more pronounced than late-season water deficits. Overall cluster weight explained most of the yield variation among the different irrigation treatments, followed by the number of berries per cluster and by the number of clusters per vine [58].

Transcriptional analysis of grape berries as well as grape berry proteome dynamics showed alterations when grape berries under moderate water deficit were compared to berries derived from well-watered vines. Alterations in the proteome dynamics, in the timing and in the amount of protein expression under moderate water deficits were observed pre-veraison and at veraison. This means that changes occurring pre-veraison due to water deficit might have implications for berry maturity [11].

In general it is presumed that berry composition is on one hand influenced indirectly by water stress through changes in the pulp/skin ratio. On the other hand different berry quality traits are also directly influenced by water deficit such as sugars, skin anthocyanin and tannin content [11]. Sugars and organic acids are the most important berry quality traits. Pre-veraison sucrose imported into the berries is mostly metabolized, whereas after veraison sugars start to accumulate in the berries. Moderate water deficit at this phenological stage enhances sugar accumulation [11]. This was also observed by Etchebarne *et al.* in Grenache noir, although interactions with leaf area treatments occurred [25]. Enhanced sugar accumulation under water deficit is either due to inhibited lateral shoot growth which leads to a redistribution of carbohydrates to the berries or due to an ABA-mediated uptake of hexose. Still the influence of water stress on sugar accumulation in the berries is dependent on the variety. This might be either due to differences in vigor (source/sink balance) or due to differences in phenological development among varieties leading to differences in phenological stages in which water stress occurs [11]. Severe water stress during berry maturation in potted plants, in contrast, showed a significant reduction in sugar accumulation in the berries compared to well-watered plants [33]. Concerning the influence of water deficit on titratable acidity results are mixed [11]. Titratable acidity is reduced under water deficit in some trials [25,94], whereas no changes in titratable acidity were observed in other trials [11,58]. Metabolization of malate in ripening berries under water deficit was observed to be higher in comparison to well-watered vines leading to a reduced ratio of malate/tartrate in berries from vines subjected to water stress [11]. This ratio, in contrast, was not affected in Grenache noir [25]. Berry mineral composition seems to be negatively affected by water stress. Cation concentration in the berries (K^+ , Na^+ , Ca^{++} , Mg^{++}) significantly decreased under water deficit compared to well-watered plants. Differences were more pronounced the more water stress occurred in non-irrigated vines [25].

Water stress influences polyphenol concentrations in berries including flavonoids, flavonols, proanthocyanins as well as stilbenes [11]. These compounds are mainly located in the endocarp and seed exocarp tissues, which are particularly known to be affected by water limitations in grapevines. On the other hand these compounds mainly located in skin and seeds of winegrapes are indirectly affected by the decrease of berry size through water stress [11]. When vine water uptake is limited before veraison, roots synthesize the phytohormone abscisic acid (ABA). ABA was found to favor anthocyanin and tannin synthesis [68]. These secondary metabolites are usually linked to high-quality red wines. Still anthocyanin composition seems to be more affected by water deficit than its total concentration in berries. Moreover, the timing of water stress seems to have major influence on anthocyanin composition in berries at harvest [11]. Berry skin color density is enhanced through late season water deficit [33]. Flavonols which serve as co-pigments with anthocyanins stabilizing red wine color do not seem to be influenced by the timing of water stress occurrence, but just by the amount of water stress. Proanthocyanidins, also known as condensed tannins, which are responsible for bitterness and astringency in wines, just seem to be slightly influenced by water deficit in grapevines [11]. Gene expression and mRNA abundance of stilbenes, which act as phytoalexins against biotic stress, were enhanced in berry skin and seeds, but no direct evidence exists for enhanced stilbene concentrations under water deficit in grapevines [11,32].

Berry aroma compounds also seem to be influenced by water deficit, although this field has not received a lot of attention in research yet. There are hints that water stress influences various wine sensory properties [11]. Cabernet Sauvignon wines subjected to water deficits were reported to have more fruity and less vegetal aromas [10].

Comparison of vineyard management systems

Vineyard management includes a huge amount of different actions and factors that have to be actively chosen by the winegrower and that influence plant performance and fruit quality of grapevines to a high extend. By choosing all these different actions the winegrower has a powerful tool to adapt the crop to different environmental factors and to determine final wine quality and taste. Vineyard management, also called the human factor in terroir, comprises the selection of the vineyard site, the variety and the rootstock, different pruning systems, trellis systems, canopy manipulation, yield reduction, irrigation, the selection of a plant protection strategy, application of herbicides or phytohormones, soil cultivation, selection of cover crops, all forms of fertilization and organic matter application [72]. A lot of research since decades concentrated on the effects of single viticultural practices such as pruning or canopy manipulation or vineyard management practices such as fertilization, under-vine management, cover cropping and pest management on grapevine performance and fruit

quality [72]. In contrast, research on the effects of different management systems on plant performance and fruit quality in viticulture are still rare. For an overview please see Chapter 5. If we consider that in the following work different vineyard management systems, as they exist today, were compared to each other, namely conventional, organic and biodynamic viticulture (Chapters 4 and 5), of course some management parameters were varied among systems, while others were kept constant.

Systems comparisons for annual cropping systems have a long tradition in agricultural research as one type of long-term field experiments [87]. One characteristic of systems comparison trials is that existing management systems are compared under realistic external conditions and furthermore they are scientifically evaluated. Several parameters are usually varied together among treatments, such as soil management, fertilization strategy, pest and disease management, application of compost and crop rotations of annual cropping systems [87]. The fact that several parameters together are varied among systems is at the same time a major advantage and a major drawback of this type of agricultural trials, because on one hand agricultural systems as they exist are compared, but on the other hand the variation of several management components at the same time makes it difficult to draw conclusions on possible reasons for changes observed. The aim of this type of field experiments is the investigation of cumulative effects on soil fertility, water and nutrient supply and yield levels [87]. The two central questions that typically arise from systems comparison trials are: (1) Do crops and do agricultural products of the different management systems differ? (2) Is the effect of the management system equal to the cumulative effect of the parameters varied within the management system or can interactions among the varied parameters be observed? Several long-term field trials comparing different management systems arose after World War II including conventional, organic and also biodynamic treatments [31,35,55,69,71]. Among these long-term field trials the most noted is the so-called 'DOK-trial' in Switzerland comparing conventional, organic and biodynamic farming including several annual crops in a crop rotation system over more than 20 years [55]. For detailed information of the effects of different management systems on annual crops please see "Research on organic versus conventional farming" and "Research on the biodynamic farming system" in Chapter 4.

In case of a systems comparison of a perennial crop such as grapevine soil management (including under-vine management, cover crop mixtures), fertilization strategy and organic matter application, pest management and application of biodynamic preparations were varied among treatments. Other management factors such as vineyard site, variety and rootstock, water supply, pruning and trellis system and vine spacing were kept constant among treatments. Canopy microclimate, fruit exposure as well as yield were not manipulated to better observe the plant performance within the compared management systems. For detailed information of the effects of these management factors please see Reynolds [72].

The origins of organic and biodynamic farming

Organic and biodynamic farming have developed at the beginning of the 20th century and are a mixture of different ideas that arose mainly in the German- and English-speaking world. Factors that influenced the development of these ideas were the biologically oriented agricultural science, the Reform movements and growing interest in the farming cultures of the Far East. The circumstances of the beginning of the 20th century, thus the crisis of agriculture between the two World Wars and the expanding chemical-technical intensification of farming, favored the development of alternative farming concepts [97].

The agricultural crisis between the two World Wars had ecological and soil-related, but also economic and social origins. The increasing application of mineral fertilizers and its consequences were discussed to be one reason for a number of ecological problems. Plant cultivars at the time were not adapted to high nitrogen levels as they were induced by the use of mineral fertilizers. At the same time the application of compost and manure was neglected and weak plants could more easily be attacked by pathogens. In addition, higher nitrogen levels in the plants led to immature seeds. These seeds of minor quality from the previous harvest could not be used any more [97]. The reason for this effect is still controversially discussed [96]. Moreover, acidic mineral fertilizers led to an acidification of the soil, which was partially wanted, but which led to reduced root growth and caused soil degradation [96]. The mechanization of agriculture caused soil compaction so that the water holding capacity of the soils was diminished. The reason for the loss of soil fertility at that time is still controversially discussed. It is likely that a disturbed balance among soil organisms led to an accumulation of harmful organic substances [97]. The dramatic yield reduction in Germany after World War I was attributed to the increasing use of mineral fertilizers. To that day it is still not clear why yields decreased as drastically. One hypothesis is that relative amounts of phosphorus were too low in relation to potassium and nitrogen amounts [96]. On the other hand consumers discussed about declining food quality, residues of plant protection agents or effects of mineral fertilization on the shelf-life of fruit and vegetables. The social and economic situation of farmers underwent a drastic change since new machinery was introduced and many people moved from the countryside to the urban centers [97].

Soil scientists started to study the soil from a biological point of view at the end of the 19th century. The biological concept of soil fertility arose. Researchers recommended feeding the edaphon by means of organic fertilization. [97] claims that “[...] organic farming is an intensification of farming by biological and ecological means in contrast to chemical intensification by mineral fertilizers and synthetic pesticides”.

Natural agriculture, part of the Life reform movement, is seen as one of the origins of organic farming in the German-speaking world. Reform movements such as the German

Life Reform (*Lebensreform*) and the American Food Reform conceived of a natural way of living rejecting industrialization and urbanization. Key concerns of these movements were protection of nature and animals, vegetarianism and self-subsistence. The reform movements and organic farming both claimed farming organically on one hand and healthy nutrition through high-quality organic food on the other hand. A minority of the followers of the Life reform movement realized their ideas about working as farmers and gardeners. Concepts such as conservation tillage, green manuring, rock powder fertilization and new composting methods were introduced by natural agriculture. Adherents of the Life reform movement rejected mineral fertilizers as well as animal manure and solely used green manuring and composting of plant residues. Natural agriculture had an association, a monthly journal and a trademark was introduced. Ewald Könemann (1899-1976) was the key person of natural agriculture and converted natural agriculture into a scientifically based organic farming system [97].

Another major source of organic agriculture in the German-speaking world is biodynamic agriculture. It was developed by Rudolf Steiner (1861-1925) in 1924 [97]. The circumstances that led to the conception of biodynamic agriculture were the same as described above. Farmers that knew his previous works invited him to speak out on farming from his anthroposophic point of view. He held his *Agricultural Lectures* ('Landwirtschaftlicher Kurs') at Koberwitz near Breslau (Silesia) in 1924 in which he proposed guidelines on biodynamic farming [83]. Based on these lectures, biodynamic agriculture was further developed by a group of anthroposophic farmers. The key concept of biodynamic farming is the concept of the farm as an organism. In this regard biodiversity plays a major role, because a wide range of plants, animals and biotopes should be part of every biodynamic farm [97]. In addition, Steiner claims the characteristic biodynamic preparations of plant and animal origin to be used (Horn manure and horn silica on one hand and the compost preparations on the other hand consisting of yarrow blossoms, chamomile blossoms, stinging nettle, oak bark, dandelion flowers and valerian flowers) [83]. Another characteristic trait of biodynamic farming is its holistic concept of nature that is conceptualized as a "[...] spiritual-physical matrix [...]" [97]. None of these principles have been adopted into science-based organic farming. Biodynamic agriculture became the focus of attention at the end of the 1920's because it displayed an alternative farming concept without the use of mineral fertilizers and claimed to produce high quality crops. Especially the followers of the agricultural sciences were against the biodynamic movement because the application of the biodynamic preparations was not in accordance with scientific principles [96]. Still today biodynamic farming practices are controversially discussed.

Natural agriculture of the Life Reform movement and biodynamic agriculture, the two main roots of organic farming in the German-speaking world, emerged almost simultaneously (Fig. 4) in similar circumstances, were both based on a biological concept of soil fertility, rejected the use of mineral fertilizers, and aimed at a sustainable form of agriculture producing high quality crops.

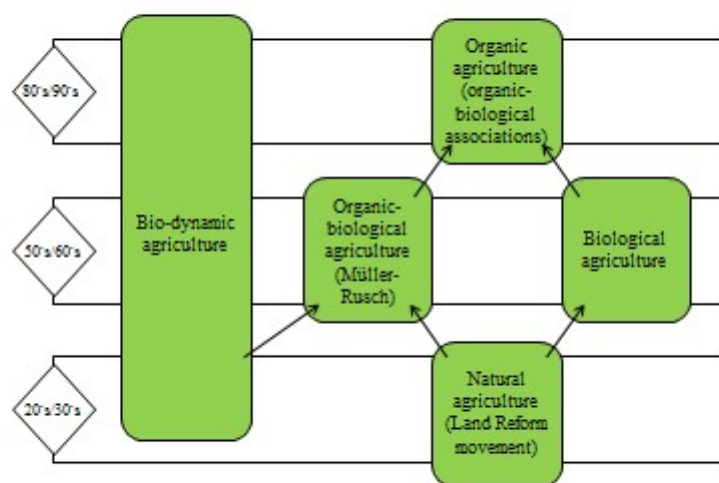


Fig. 4: Development of different forms of organic agriculture in the German-speaking world throughout the 20th century (modified according to [96]).

Besides that they disagreed in some essentials such as animal husbandry and composting. The most important difference between the two concepts is that natural agriculture is based on scientific principles of soil science and ecology, whereas biodynamic farming is rooted in the anthroposophic view of nature [96]. This is still true today for the concepts of organic farming on one hand and biodynamic farming on the other hand.

Origins of organic farming in the English-speaking world

The beginnings of organic farming in the English-speaking world lie in India. Here two pioneers of organic farming, one agricultural scientist, Albert Howard (1873-1947), and one medic, Robert McCarrison (1878-1960), have been working. Albert Howard, together with his first and then with his second wife, laid the foundation of organic farming in the UK as well as in the USA. They worked on composting techniques and on using urban organic waste to maintain soil fertility. Furthermore, they emphasized the importance of soil fertility as the starting point for ensuring plant, animal and human health. As a doctor, Robert McCarrison studied the link among soil fertility, food quality and human nutrition. He concentrated on examining the conditions determining human health rather than to cure diseases and was of the strong opinion that “[...]”

properly composted organic residues will create a fertile soil, on which strong plants will grow, offering a healthy diet for humans and animals” [97]. He also worked on the effect of synthetic mineral fertilizers on food quality and human nutrition and health.

Organic farming concepts that were developed in the UK were strongly influenced by Albert Howard’s ideas and were similar to those developed in Germany. Based on the biological concept of soil fertility mentioned above a lot of new soil management techniques were introduced, e.g. ploughless soil cultivation, organic soil cover, green manuring and ley farming. The British organic farming association The Soil Association was founded in the 1940’s by Eve Balfour (1898-1990). The Haughley experiment, the first long-term experiment on organic farming, was set up by her.

The beginnings of organic farming in the USA were linked to wind erosion at the start of the 20th century that seriously damaged the soil in the Great Plains. A group of scientists promoted a sustainable way of farming preventing soil erosion. Several ploughless soil cultivation methods were introduced using organic soil covers. Similar to the Life Reform movement in Germany, the Food Reform movement in the US promoted a vegetarian lifestyle, back-to-the-land initiatives and organic gardening [97]. The American editor Jerome I. Rodale (1898-1971) played a key role within the movement. In 1947 he founded the Soil and Health foundation (Rodale Institute) for developing practical methods to enhance soil fertility in farming and to promote healthy diets [61]. One of the early long-term trials comparing organic and chemical agriculture was established here [35].

Further evolution of organic and biodynamic farming

The original concepts of natural and biodynamic agriculture further evolved after World War II (Fig. 3). Improvements in mechanization and the usage of new crop hybrids, fertilizers and pesticides led to a high increase in farm productivity (‘Green Revolution’), but some consequences of modern industrial agriculture arose by the 1950’s. Environmental pollution was observed and resistances against insecticides began to develop. In 1962 Rachel Carson’s book *Silent Spring* was published. The publication of this book is considered the starting point for the environmental movement not only in the US [61,97].

During the evolution of organic farming in the German-speaking world some key principles of natural agriculture such as vegetarianism and farming without animals have been abandoned. In contrast, scientific concepts of biologically stabilized soil structure and rhizosphere dynamics were included and led to a science-based form of organic farming called biological agriculture during the 1950’s and 1960’s [97]. Furthermore, new agricultural techniques and tools concerning soil management and animal husbandry were developed explicitly for organic farming [30,47].

In parallel to biological agriculture the organic farming practice of organic-biological agriculture was developed in Switzerland, Austria and Germany at the same time by combining traditional agricultural techniques with aspects of natural agriculture, biodynamic farming and experiences of British organic farming (Fig. 4) [97]. Ideas were mainly developed by the married couple Hans and Maria Müller and by Hans Peter Rusch [74,75]. Organic-biological farming was characterized through ley farming, sheet composting and conservation tillage. Rural culture and Christian faith also played a key role during the 1950's and 1960's [97].

Organic-biological agriculture as well as biological agriculture merged during the 1970's in Germany to become today's organic farming (called ecological agriculture in Germany) adopting science-based concepts of agriculture developed in the previous decades [97]. During the 1980's associations and other professional organizations arose which established certification and marketing of organically produced agricultural crops [97]. The first production standards concerning organic viticulture in Germany were introduced in 1985. In 1991 the first EU-wide regulation on organic production of agricultural products was established (Council Regulation (EC) No 2092/91). State subsidies were associated with it and this, together with other aspects, led to an increase of organically managed agricultural surface (see 3.5) [3,45]. During the 1980's and 1990's environmental protection played a key role within the organic movement [97].

First pioneers of organic and biodynamic viticulture started to develop an ecologically oriented approach of viticulture focused on biodiversity in the monoculture of the vineyard. This was mainly caused by the 'Green Revolution' in agriculture starting during the 1960's using high-yielded crops, increasing amounts of mineral fertilizers and synthetic plant protection agents and improved mechanization and crop hybrids [17,61]. In the 1980's the first rules on organic viticulture were elaborated and were established in Geisenheim in 1985 [45]. At the same time the German Organic Winemaking Association (Bundesverband Ökologischer Weinbau BÖW), today called ECOVIN Bundesverband Ökologischer Weinbau, developed and agreed on unifying national guidelines for Germany [17,45]. These guidelines included rules on viticultural aspects as well as on oenological practices [17]. The EU-wide regulation on organic production of agricultural products (Council Regulation (EC) 2092/91) was established in 1991 and affected organic viticulture as well. Control mechanisms became government-regulated and state subsidies were introduced [17,45].

The evolution of biodynamic agriculture after Steiner having outlined its principles in his lectures in 1924, occurred mainly on vast estates spread throughout pre-World War II Germany. Activities concentrated on manuring, breeding, animal husbandry and the application of the biodynamic preparations suggested by Steiner. Soon after Steiners lectures on agriculture an experimental group, regional associations and marketing cooperatives developed. The trademark Demeter was established in 1928 [97].

During the Third Reich biodynamic organizations were allowed to continue working. Some Nazi officials were interested in testing the potential of biodynamic agriculture regarding sustainability, food quality and self-sufficiency concerning fertilizers. In 1941 anthroposophic and biodynamic associations were forbidden [97].

The new situation after World War II required changes also in biodynamic farming practices and concepts. During the 1950's and 1960's scientific concepts were integrated into biodynamic agriculture. The Forschungsring für Biologisch-Dynamische Wirtschaftsweise was founded in 1946 [97]. Research concentrated on the effect of biodynamic preparations, influence of celestial bodies and food quality of biodynamic crops [48]. This was the period when the first scientific trials on the comparison of biodynamic and organic or conventional agriculture were established in Sweden and afterwards in Switzerland and Germany [31,55,70]. From the 1980's on the focus of biodynamic research changed from proving the effectiveness of biodynamics towards solving specific questions concerning the development of biodynamic agriculture [48]. Specific methods for quality control of agricultural crops such as biocrystallization newly gained attention [41,100].

Legislative Regulations for organic and biodynamic viticulture in Europe today

The guidelines of the *code of good practice* (Gute fachliche Praxis) are compulsive for all winegrowers in Germany. Furthermore, the Federal Soil Protection Act (Bundes-Bodenschutzgesetz BBodSchG), the German Fertilizer Ordinance (Düngeverordnung DüV), the European Water Framework Directive (Europäische Wasserrahmenrichtlinie), the Biowaste Ordinance (Bioabfallverordnung BioAbfV) and the German Plant Protection Act (Pflanzenschutzgesetz PflSchG) have to be respected by all agricultural producers [45]. Organic as well as conventional winegrowers use integrated pest management (IPM) strategies for pest and disease control. Field monitoring for pest and predator numbers and the use of risk assessment models are essential techniques of IPM. Threshold levels are established to guide decisions of pest and disease control. Cultural practices such as defoliation of the bunch zone for Botrytis prevention and better application of fungicides are another important part of the IPM strategy [62].

Beyond that there are three European Regulations that are relevant for organic and biodynamic winegrowers in Germany. The Council Regulation (EC) No. 834/07 lays down aims and principles of organic farming as well as instructions on production, labelling, control and trade of organically produced agricultural crops. The Council Regulation (EC) No. 889/08 contains detailed information about the implementation of these principles and instructions including lists of allowed fertilizers and plant

protection agents in organic farming (Annex I and II, respectively). The Regulation (EC) No. 203/12 is a guideline for organic wine production relevant for products labelled as organically certified. It includes a positive list of additives and treatments allowed for organic wine production. The Regulation (EC) No. 491/09 (Weingesetz) and No. 606/09 again are relevant for all German winegrowers [37,45,85].

The practice of organic and biodynamic viticulture in Germany today

Practice of organic and biodynamic viticulture today might be even more dependent on environmental factors than conventional or integrated viticulture. Since the use of synthetic pesticides and herbicides and the application of mineral fertilizers in organic and biodynamic viticulture is forbidden worldwide, these viticultural systems have to be well-adapted to the environmental conditions, maybe even more than their conventional or integrated counterparts. (For a short overview of legislative regulations of organic viticulture worldwide see Chapter 4).

Viticultural parameters that can help adapting the vineyard to environmental conditions are the selection of vineyard site, variety and rootstock, pruning and trellis system and the regulation of growth and vigor. Especially climatic conditions concerning precipitation and its distribution throughout the growing season are important parameters for further adaptation of the vineyard, because the plant protection strategy in organic and biodynamic viticulture is one crucial point, especially in cooler climates. In this context the regulation of growth and vigor influencing the light exposure and the ventilation of canopy and grapes in the bunch zone plays a major role for ensuring a successful organic management of the vineyard. Further regulation of vigor and leaf area to fruit weight ratio can be done by green shoot removal, defoliation or yield reduction. New hybrid varieties tolerant against downy and powdery mildew (*Plasmopara viticola*, *Uncinula necator*) might help making the vineyard even more adapted to organic or biodynamic viticulture in comparison to traditional varieties that are often very susceptible against these fungal diseases [38,45].

Water and nutrient supply of the vines and thus growth and fruit ripening is highly dependent on soil management, especially in organic and biodynamic viticulture where no herbicides and no mineral fertilizers are applied. A soil management adapted to climatic conditions on one hand is crucial in organic viticulture to prevent soil depletion, erosion and soil compaction. On the other hand soil management has to meet the needs of the vines concerning nutrient and water availability in the different growth stages. A central aim of organic soil management is to increase soil fertility by increasing the organic matter content. Cover crops play an important role within soil management strategies in organic and biodynamic viticulture. By selecting different

cover crop mixtures for different climatic conditions biodiversity in the vineyard can be increased, soil erosion and compaction can be prevented, nutrient supply is ensured and soil life is enhanced. The continuous monitoring of the soil status is a very important tool in organic farming in general to ensure optimal adaptation of the agricultural system to environmental conditions [38,45].

The usage of cover crop mixtures is highly linked to the soil type, the soil water-holding capacity and the distribution of precipitation throughout the growing season. Even under cool climate conditions in Germany strategies can vary from a complete green cover within the rows to the use of winter cover crops combined with ploughed soil throughout the summer in case water availability is restricted during the growing season. One characteristic strategy for german organic viticulture includes an alternating soil management of every second row to ensure nutrient supply of the vines at full-bloom, guarantee a green cover of every second row during the growing season for carrying out plant protection treatments, and benefit from all the other advantages of cover crops (prevention of soil erosion, improvement of soil structure, increase of biodiversity) [45] (Fig. 5).

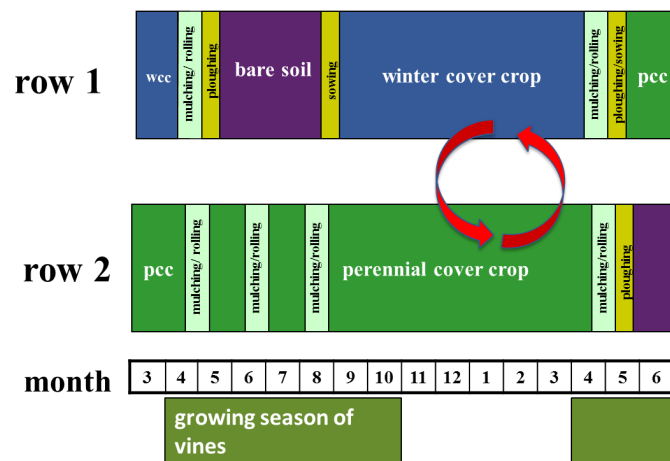


Fig. 5: Strategy of a perennial soil management system under cool-climate conditions (modified according to [45])

In general cover crop mixtures are used in organic and biodynamic viticulture because of an increase in biodiversity. According to their duration we can differentiate among winter cover crops, annual or perennial cover crop mixtures. Of course the root depth and the water demand of the cover crop mixture has to be adapted to the specific environmental conditions of every single vineyard site. Cover crop mixtures rich in legumes (Fig. 6) play a major role in organic and biodynamic viticulture in Germany

because by working these cover crops in spring nitrogen supply of the vines can be ensured [45].



Fig. 6: Cover crop mixture rich in legumes characteristic for cool climate organic viticulture (Picture: Döring).

By mulching or rolling water consumption of cover crops can be regulated throughout the season [38,45] (Fig. 7).



Fig. 7: Visible effects of mulching and rolling of green cover in viticulture (Picture: Kauer).

Under-vine management in organic and biodynamic viticulture is of major importance since the application of chemical herbicides is forbidden in organic agriculture. It is important to control plant growth in the under-vine area to ensure sufficient water

supply of the vines. Three major strategies for under-vine management consist of mechanical cultivation, under-vine cover cropping or usage of different covers such as straw. The most common strategy in Germany is soil cultivation in the under-vine area either with a flat share or with a disc [38].

The concept of fertilization in organic agriculture differs substantially from the one of conventional agriculture, where plants are directly supplied with inorganic fertilizers to displace nutrients in the soil. In organic farming plants should be enabled to actively take up nutrients from the soil by enhancing soil fertility and soil life and thus promoting soil nutrient cycling by soil macro- and microorganisms [76]. If there is a serious nutrient deficiency, fertilizers listed in Regulation (EC) No. 889/08 Annex I can be used. Beyond that the German Fertilizer Ordinance (Düngeverordnung DüV), the European Water Framework Directive (Europäische Wasserrahmenrichtlinie) and the Biowaste Ordinance (Bioabfallverordnung BioAbfV) have to be respected. Farm manure as well as secondary raw fertilizers (e.g. green waste compost), commercial organic manures, chalks and mineral fertilizers containing magnesium, phosphorus, potassium and trace elements can be used in organic farming [38,45].

Plant protection is one of the most challenging aspects of organic viticulture under cool climate conditions, since traditional varieties are highly susceptible against downy and powdery mildew (*Plasmopara viticola*, *Uncinula necator*) and wet conditions during the growing season favor the propagation of downy mildew. Several plant protection agents listed in Regulation (EC) No. 889/08 Annex II can be used in organic viticulture. There are several groups of substances listed in Annex II of the respective Regulation (EC): Substances of crop or animal origin, micro-organisms (e.g. *Bacillus thuringiensis*) and substances produced by micro-organisms, substances to be used in traps or dispensers (pheromones), surface-spread substances, other substances of traditional use in organic farming (copper, sulfur, mineral oils) and other substances (potassium bicarbonate). Moreover, the German Plant Protection Act (Pflanzenschutzgesetz PflSchG) has to be respected. This has implications especially for the use of copper (max. 3 kg/ha and year in Germany vs. max. 6 kg/ha and year for EU). The most relevant protection agents in organic viticulture are copper, sulfur, potassium bicarbonate and pheromones or products containing *Bacillus thuringiensis*. The use of several additives is also allowed in organic viticulture. Plant resistance improvers were widely used in organic viticulture in Germany several years ago, but changes in the definition of plant protection agents on the EU level and in the definition of plant resistance improvers within the German Plant Protection Act reduced their relevance and usage [38,45].

All the described principles and practices of organic viticulture are also valid for biodynamic viticulture. Beyond that, organic and biodynamic viticulture differ substantially in their concept of nature and agriculture, as described in section 3.2. Concerning the practices one major difference between the two management systems is

the application of the characteristic biodynamic preparations in biodynamic viticulture. They consist of field spray and compost preparations. In most of the cases medicinal herbs are put into animal envelopes and buried under the ground for six months. These preparations are then either stirred in water in small quantities and then sprayed on soil or vines or applied to the compost. Horn manure and horn silica (Fig. 8) are the two most known preparations [56].



Fig. 8: The making of the horn silica preparation before it is buried below ground in spring (Picture: Döring).

Horn manure in viticulture is characteristically applied to the soil once or twice in spring and once after harvest, whereas horn silica is applied three to four times during the growing season coupled to different phenological stages of the vines [56].

The compost preparations consist of yarrow blossoms (Fig. 9), chamomile, stinging nettle, oak bark, dandelion and valerian [56].



Fig. 9: The compost preparation consistent of yarrow blossoms (*Achillea millefolium*) aged below ground during the winter months (Picture: Döring).

Beyond the application of the biodynamic preparations teas and plant extracts are used in biodynamic farming and viticulture. On one hand they are used to regulate growth, on the other hand they protect plants from biotic and abiotic stresses in addition to the methods and agents used in organic viticulture [56].

The respect and the inclusion of natural rhythms into the everyday work is another essential characteristic of biodynamic viticulture and farming. This is relevant for the timing of the application of biodynamic preparations as well as for the timing of the different types of work carried out in the vineyard throughout the year [56].

A detailed overview of the effects of organic and biodynamic viticulture in opposition to integrated and conventional viticulture can be found within the systematic quantitative review in Chapter 5.

Importance of organic and biodynamic viticulture today

Grapes are among the most important permanent crops worldwide. Permanent cropland has a higher share of organic production compared to arable farming. The share of organic production for perennial crops is 8 % [50]. Most of this organically managed cropland lies in Europe. By 2014 316'000 ha or 4.5 % of the grape growing area worldwide were managed according to organic standards. Again most of this organically managed viticultural surface (over 80 %) lies within Europe (266'000 ha or 6.8 %), but not the complete surface is used for wine grape production. Since 2004 when the first assessment took place, the grape growing surface used for organic production has more than tripled (Fig. 10).

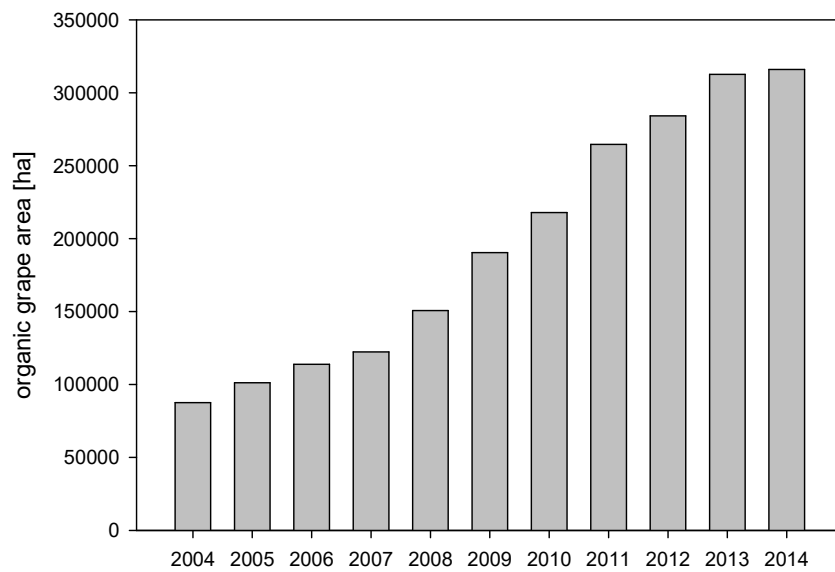


Fig. 10: Development of the grape growing area worldwide managed according to organic standards (modified according to [50]).

The countries with the largest grape area under organic management are Spain, Italy and France. These countries also have the highest share of organically managed viticultural surface (Table 1).

Table 1: Organic grape area and organic share of selected countries in 2014 (modified according to [50]).

| country | organic area [ha] | organic share [%] |
|--------------------------|-------------------|-------------------|
| Argentina | 3466 | 1.5 |
| Australia | 282 | 0.2 |
| Austria | 4677 | 10.7 |
| China | 15729 | 2.1 |
| France | 66211 | 8.7 |
| Germany | 7500 | 7.5 |
| Italy | 72361 | 10.3 |
| South Africa | 1056 | 0.8 |
| Spain | 84381 | 8.9 |
| United States of America | 15647 | 4 |

Germany had 7500 ha of organically managed viticultural surface in 2014, which is a share of 7.5 % of the whole grape growing area [50].

The evolution of the organic grape growing area described above could also be observed for organic farming and organic viticulture in Germany. The introduction of the first EU-wide standards for organic production of agricultural products (Council Regulation (EC) No 2092/91) in 1991 played a key role, as mentioned before [45]. Since the year 2000 a substantial growth of the number of farms working organically as well as a substantial growth of the agricultural surface managed according to organic standards could be observed in Germany (Fig. 11) [3].

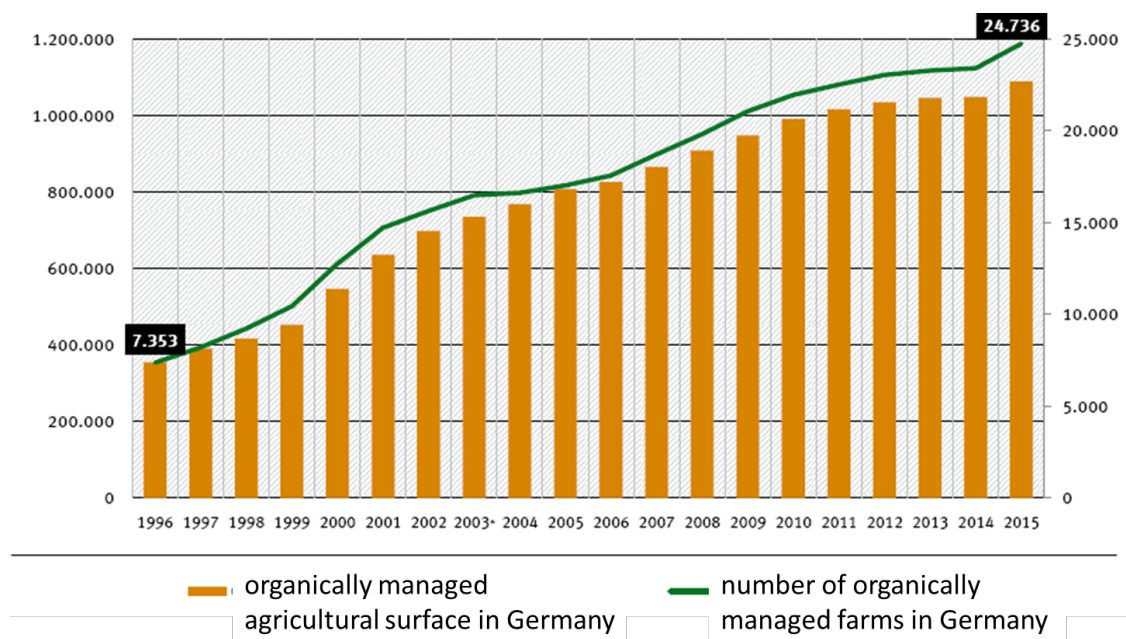


Fig. 11: Number of organically managed farms and agricultural surface managed according to organic standards in Germany from 1996 to 2015 (modified according to [90]).

In 2006 825.539 ha of agricultural land in Germany were managed according to organic standards, which is 4.9 % of the whole agricultural surface in Germany at the time [3,49]. At the end of 2016 1.251.320 ha of agricultural land in Germany were managed according to organic standards. That corresponds to a share of 7.5 % of the whole agricultural surface in Germany [49]. Two third of the organically certified producers in Germany were certified according to EU standards and belonged to one of the organic associations at the end of 2016, whereas about one third of the german organic producers were only certified according to EU standards [65].

The biggest german association for organic viticulture is ECOVIN. ECOVIN was founded in 1985 and had 35 members at the time [23]. The whole organically managed viticultural surface in Germany in 1985 was 150 ha [45]. At the end of 2016 ECOVIN had 236 members who managed 2380 ha of vineyards according to organic standards in

Germany [23]. Bioland, Naturland and Demeter are other important associations where organic winegrowers as well as organic farmers are organized. Demeter e.V. is the oldest organic association in Germany being founded in 1924. Demeter e.V. today includes about 1500 farms that manage more than 77800 ha according to biodynamic standards in Germany, among them 58 winegrowers [22]. Demeter is an international association. Worldwide there are 616 biodynamic winegrowers working according to Demeter standards and managing a surface of more than 8200 ha of vineyards [22]. Bioland e.V. was founded in 1971 as “bio gemüse e.V.”. In 2000 121720 ha (3561 farms) in Germany were managed according to Bioland standards, whereas in 2017 343489 ha (6861 farms) belonged to the association Bioland [26]. In 2013 126 wineries were certified according to Bioland standards in Germany, corresponding to 1025 ha of vineyards (Fig. 6) [45]. About half of the german organically managed viticultural surface (3806 ha) was certified according to EU standards as well as to standards of organic associations, whereas the other half was organically certified according to EU standards only (3594 ha) in 2013 [45].

Objectives

This Ph.D. project was performed to investigate the influence of the ‘terroir’ factors soil water-holding capacity and grapevine genotype on plant performance on one hand and the influence of organic and biodynamic management on plant performance and fruit quality of grapevines on the other hand. In order to assess influences on plant performance of grapevines a new, reliable and quick method for leaf area index assessment in VSP trained vineyards was established. The Ph.D. project was subdivided into four parts to achieve the respective aims:

Leaf area to fruit weight-ratio is one of the most relevant measures for plant performance and crop load influencing winegrape quality to a very high extend [46]. In viticulture different approaches for leaf area assessment exist, but they either require a lot of equipment or are very time-consuming [5,51]. The first objective of this doctoral dissertation was to establish a reliable, fast and accurate method to non-destructively and dynamically assess leaf area which could be applied in a small scale in the field. Since the assessment of plant performance typically requires non-destructive measurements of leaf area in the field throughout the growing season, different protocols for indirect estimation of leaf area index (LAI) by gap fraction analysis in VSP trained grapevines (*Vitis vinifera* L. cv. Riesling) were tested. Measurements were carried out using the portable Plant Canopy Analyzer (PCA, LAI-2200, LI-COR, Lincoln, NE, USA). Results are presented in Chapter 2.

Soil and cultivar are two major ‘terroir’ factors influencing plant performance and fruit quality, as described in the introduction. The interaction between the influence of

grapevine genotype and soil water-holding capacity on plant physiological parameters was assessed in the following Chapter to accurately describe the contribution of these two 'terroir' factors to plant performance. Two different cultivars of *Vitis vinifera* L. were selected, one displaying a more near-isohydric (Cabernet Sauvignon) and one showing a more near-anisohydric (Syrah) response to water stress. Two different substrates with different water-holding capacities were used to characterize hydraulic and stomatal responses of the two different grapevine genotypes. The trial was set up with potted vines in a greenhouse to control water uptake by the plants. Stem-water potentials together with percent loss of conductivity (PLC) were determined to describe physiological response mechanisms of the two different grapevine genotypes on these two soil substrates with different water-holding characteristics. Embolism formation and repair are closely linked to stomatal control and thus to chemical root-to-shoot signaling of the plants. For further description of the plant's reaction to water deprivation stomatal conductance and ABA content in leaves inducing stomatal closure and thus controlling water loss were determined. Results are described in Chapter 3.

Organic and biodynamic management systems are gaining more and more importance in the wine sector. Especially in the last two decades the viticultural surface being managed according to organic or biodynamic standards has increased substantially. Reliable field trials assessing the effects of organic and biodynamic management practices in viticulture are rare. One major aim of this doctoral dissertation was to describe the impact of organic and biodynamic management on grapevine performance and fruit quality compared to integrated management. Data of a field trial (*Vitis vinifera* L. cv. Riesling) comparing organic, biodynamic and integrated viticulture in Geisenheim, Rheingau, were collected over a three-year period (2010-2012) after conversion to characterize the effects of the respective management systems on growth, yield and fruit quality. The integrated treatment was managed according to the *code of good practice* [6]. Organic and biodynamic plots were managed according to Regulation (EC) No 834/2007 and Regulation (EC) No 889/2008 and according to ECOVIN- and Demeter-Standards, respectively. By assessing different key parameters for plant performance and fruit quality such as pre-dawn water potential, physiological performance, leaf area to fruit weight-ratio and disease incidence hypotheses concerning the reasons for the observed changes are presented. Results are displayed in Chapter 4.

In addition, a systematic quantitative review evaluating the effects of organic and biodynamic viticulture worldwide was done. Influences of the management systems on soil parameters, biodiversity, vine growth and yield, disease incidence, grape composition and wine quality as well as on sensory characteristics of the wines were summarized and evaluated taking into consideration available literature on the topic including field trials as well as surveys done in commercial vineyards or with commercial wines. By this systematic quantitative review overall effects of organic and biodynamic viticulture irrespectively of environmental factors were described and results are presented in Chapter 5.

Chapter 2 Indirect Estimation of Leaf Area Index in VSP-Trained Grapevines Using Plant Area Index¹

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Technical Brief

Indirect Estimation of Leaf Area Index in VSP-Trained Grapevines Using Plant Area Index

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and Susanne Tittmann¹

Abstract: Leaf area index (LAI) and canopy structure are important parameters affecting grape quality and yield of grapevines. Two different experimental protocols as well as the average LAI value of the different protocols for indirect estimation of LAI by gap fraction analysis in VSP-trained grapevines (*Vitis vinifera* L. cv. Riesling) were tested in this study using plant area index (PAI). Measurements were performed using a plant canopy analyzer. Directly measured LAI and estimated PAI were compared. Protocol SFC (sensor facing the canopy) gave accurate estimates of LAI by measuring PAI along a diagonal transect including eight vines on each side. The correlation between directly measured LAI and estimated PAI was very high ($R^2 = 0.93$) and the root mean square error was lowest of the methods tested here (RMSE = 0.21). Eight measurements below the canopy were enough to accurately estimate LAI. By applying the empirical calibration equation, the measurements provide accurate LAI estimates. Nevertheless, local calibration is required. The method presented provides a useful tool for rapid and precise LAI estimation in VSP training systems and for supporting canopy or management decisions based on LAI.

Key words: leaf area, leaf area index, plant area index, gap fraction analysis, grapevine

Rapid, nondestructive, and accurate measurement of leaf area is of central importance in agronomic, physiological, and ecological studies (Montero et al. 2000). Leaf area is one of the most important indicators of plant growth and vigor. Canopy structure and leaf area may reveal a plant's short-term and long-term adaptation to different environmental factors because they determine photosynthetic and evaporation rates (Norman and Campbell 1989, Eschenbach and Kappen 1996). Thus, leaf area is frequently used to compare canopy development or structure over time, under differing environmental conditions, among species, or between varieties (Martens et al. 1993). In viticulture leaf area is a crucial indicator of water use, whole-plant assimilation, light interception, and impact on bunch exposure. Growth and vigor mainly influence vine microclimate and hence fruit quality. Therefore, reliable measurements of leaf area and leaf area

index (LAI) are of importance in commercial winemaking and viticultural field studies.

Direct measurement of leaf area based on destructive harvests is usually not desired. Direct, nondestructive determination of leaf area is tedious and time-consuming, requires multiple replicates to reduce sampling errors (Mabrouk and Carbonneau 1996, Montero et al. 2000, Lopes and Pinto 2005), and several models are not applicable for hedged canopies (Mabrouk and Carbonneau 1996, Lopes and Pinto 2005). Indirect techniques of LAI estimation that are based on the relation between radiation interception and canopy structure such as measurements of canopy gap fractions provide an alternative (López-Lozano and Casterad 2013). The probability of light penetration depends on foliage distribution in space, angular distribution of foliage elements, and the angle of incoming light (Welles and Norman 1991). There are different commercial instruments available based on canopy structure measurements by gap fraction analysis, including the LAI-2200 Plant Canopy Analyzer (PCA). The theory of operation has been evaluated in detail (Welles and Norman 1991, LICOR 2009). One of the assumptions of gap fraction analysis is randomly distributed foliage. Performance of the PCA under experimental conditions has been mediocre. Determination of LAI in homogeneous crop canopies such as soybean and prairie grasses was accurate and reproducible (Welles and Norman 1991), but direct LAI was underestimated even in forests where random distribution of foliage is assumed (Chason et al. 1991, Eschenbach and Kappen 1996).

Many studies show the difficulties of implementation of gap fraction analysis in row crops and trellised vineyards in particular (Grantz et al. 1993, Grantz and Williams 1993, Sommer and Lang 1994, Watanabe et al. 1997, Ollat et al. 1998, Patakas and Noitsakis 1999, Cohen et al. 2000, Johnson

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and Pierce 2004) due to the heterogeneous distribution of foliage in rows. One protocol designed for vineyards underestimated actual LAI and resulted in a curvilinear relationship (Grantz and Williams 1993) and a similar protocol underestimated LAI in cordon-trained grapevines (Patakas and Noitsakis 1999). Two different measuring protocols were applied to minimal- and spur-pruned grapevines using a PCA and both underestimated LAI (Sommer and Lang 1994). Watanabe et al. (1997) compared directly measured and estimated LAI in fully developed *Vitis labrusca* L. cv. Concord and *Vitis vinifera* L. cv. Chardonnay canopies and recommended a 45° view angle for narrow canopies and a view direction to the row. A two-azimuth protocol allowed accurate estimation of LAI in a vertical shoot-positioned (VSP)-trained vineyard, although the coefficient of determination between actual and measured LAI was low ($R^2 = 0.76$) (Ollat et al. 1998). A two-azimuth protocol applied to different viticultural training systems resulted in a linear relationship between actual and measured LAI, but again showed a rather weak relationship ($R^2 = 0.78$) (Johnson and Pierce 2004). One major characteristic of the measurements of canopy gap fractions is that it is not possible to distinguish between photosynthetically active plant tissue and other plant elements or trellis structures. Therefore, alternative terms have been proposed, such as plant area index (PAI) (Neumann et al. 1989, Sommer and Lang 1994, Patakas and Noitsakis 1999). However, the applicability of gap fraction analysis in grapevine has not yet been investigated satisfactorily, as no measuring protocol with high coefficient of determination that accurately estimates LAI is available for VSP-trained grapevines. Successful determination of LAI would greatly facilitate the study of grapevine growth and vigor and would help estimating the photosynthetically active plant tissue as an important ecophysiological parameter in grapevine field studies.

The aim of this study was to compare directly measured LAI and estimated PAI for VSP-trained grapevines with respect to the influence of trellis structures and other plant elements on gap fraction analysis. Another objective was to design a protocol that accurately and rapidly estimates LAI in a VSP-trained vineyard (*Vitis vinifera* L. cv. Riesling) by using a PCA and to draw conclusions on the applicability of gap fraction analysis in row crops.

Materials and Methods

The experiments were performed at Geisenheim, Germany (49°59'N; 7°56'E), in a vineyard planted in 1991 (*V. vinifera* Riesling clone Gm 198-30, grafted on *V. berlandieri* Planch. x *V. riparia* Michx. cv. SO4 and *V. riparia* Michx. x *V. cinerea* Engelm. cv. Börner). The vines had a spacing of 1.2 m within rows and 2 m between rows using a VSP system and were cane-pruned (5 nodes/m²). Rows were oriented north to south. The distance from soil to canopy was ~87 cm and canopy height of the entirely developed canopy was ~133 cm on average.

Indirect measurements of PAI were conducted using a LAI-2200 Plant Canopy Analyzer (PCA; LAI-2200, LI-COR, Lincoln, NE). The optical sensor of the PCA incorporates fisheye optics to project a hemispheric image onto

five concentric rings that simultaneously measure penetration of diffuse radiation at different zenith angles (mean zenith angles of 7°, 23°, 38°, 53°, and 68°, respectively). The technique combines a measurement of sky brightness from above the canopy with measurements below the canopy. The ratio of each ring's signals (below to above reading) is assumed to be equivalent to the gap fraction of the canopy at the specific viewing angle (Welles and Norman 1991). The below-canopy measurements are combined by averaging the logarithms of the computed gap fractions (Lang and Xiang 1986). Gap fractions are then converted to LAI (Miller 1967). The instrument includes a filter to limit the radiation spectrum to <490 nm. Measurements were taken under diffuse light conditions (either during the day under cloudy sky conditions or during the last hour before sunset under clear sky conditions). A LI-COR view cap covering 315° of the azimuthal field of view was used after performing the gap test, as recommended by the manufacturer (LI-COR 2009).

Indirect estimates of PAI were obtained using two different protocols along a diagonal transect within two adjacent rows including eight vines on each side. The relationship between LAI estimation with LAI-2000 (same measuring principles as LAI-2200) and direct LAI for single vines in vineyard rows has been shown as weak (Ollat et al. 1998). Hence, groups of four adjacent vines in two adjacent vineyard rows were taken into consideration as the smallest unit for assessing the relationship between estimated PAI and directly measured LAI. For one protocol the measures were obtained with the sensor facing the canopy (SFC); for the other protocol the measures were performed with the sensor viewing along the row (SAR), according to Welles and Norman (1991) and the manufacturer's guidelines for row crops (LI-COR 2009). For the two protocols, one reading above the canopy (A-reading) was followed by 29 readings below the canopy (B-readings) at 30 cm intervals to obtain accurate measures (LI-COR 2009). The development of the estimated PAI along the transect differs substantially between the two protocols SFC and SAR (Figure 1). B-readings were obtained at 20 cm aboveground. The day after the indirect measurements of PAI the four central vines of each row within the transect were defoliated and the direct LAI was measured using a LI-3100 Area Meter (LI-COR). The calibration was performed by progressively removing leaves from the canopy, ~20% of the canopy each time. Measurements were conducted from May to October 2012 at four different phenological stages (modified E–L 15, 29, 35, 38, determined according to Coombe 1995).

Linear regression analysis was used to evaluate the quality of the correlation between directly measured LAI and estimated PAI. Analysis of covariance was performed for comparison of regression equations. All statistics were carried out using the statistical software R (Ihaka and Gentleman 1996).

Results

The number of necessary readings below the canopy was assessed. The coefficient of determination (R^2) and the root mean square error (RMSE) for the two different protocols and

for the average value of the two protocols were determined depending on the number of below-canopy readings (Table 1). Equally distributed points along the transect were chosen when the number of readings below canopy was reduced, taking into consideration the first and the last measuring point of the transect. Eight readings per measured transect below canopy at ~118 cm intervals still gave accurate results compared to the actual number of 29 readings below canopy. The reduction of B-readings neither affected negatively the

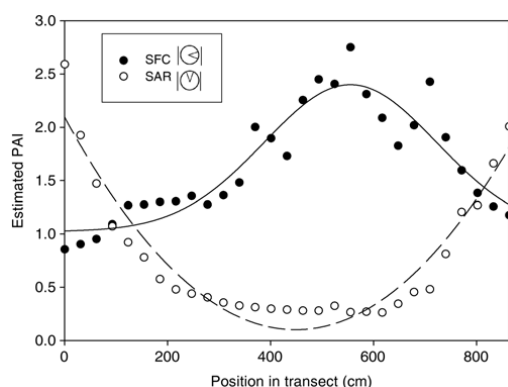


Figure 1 Estimated PAI for every single measuring point along the transect for protocol SFC (sensor facing the canopy) and protocol SAR (sensor along the row). Full line represents the regression curve (Gaussian) for protocol SFC ($R^2 = 0.87$) and dashed line represents the regression curve (Gaussian) for protocol SAR ($R^2 = 0.92$).

Table 1 Coefficient of determination (R^2) and root mean square error (RMSE) of two experimental protocols, SFC (sensor facing the canopy) and SAR (sensor along the row), and average of both protocols using different numbers of readings below the canopy (B-readings).

| Measuring protocol | B-readings (n) | R^2 | RMSE |
|--------------------|----------------|-------|------|
| SFC | 29 | 0.93 | 0.22 |
| | 15 | 0.93 | 0.21 |
| | 8 | 0.93 | 0.21 |
| | 5 | 0.88 | 0.27 |
| | 3 | 0.82 | 0.39 |
| | 2 | 0.76 | 0.57 |
| SAR | 29 | 0.61 | 0.69 |
| | 15 | 0.63 | 0.69 |
| | 8 | 0.72 | 0.65 |
| | 5 | 0.66 | 0.53 |
| | 3 | 0.65 | 0.55 |
| | 2 | 0.61 | 0.72 |
| Average | 29 | 0.97 | 0.37 |
| | 15 | 0.97 | 0.39 |
| | 8 | 0.97 | 0.39 |
| | 5 | 0.92 | 0.37 |
| | 3 | 0.86 | 0.38 |
| | 2 | 0.75 | 0.41 |

quality of the correlation between directly measured LAI and estimated PAI nor affected substantially the RMSE for all three measuring protocols. Further reduction of the number of B-readings resulted in a decrease of the coefficient of determination (R^2) for the two different experimental protocols and for the average PAI values of the two protocols. For protocol SFC (sensor facing the canopy), the RMSE was lowest of all tested methods and increased substantially when the number of B-readings was reduced from eight to five (Figure 2). For protocol SAR (sensor along the row), the RMSE was high and constantly decreased when the number of B-readings was reduced from 29 to five and it was high and remained nearly constant for the average PAI values of the two experimental protocols.

PAI estimation through protocol SFC showed a high correlation with directly measured LAI and approximated the 1:1 correlation expressed as the lowest RMSE of the tested methods (Figure 3A). By applying protocol SAR, the correlation was weaker and directly measured LAI was strongly underestimated, resulting in a high RMSE (Figure 3B). With protocol SAR, the coefficient of determination became slightly higher with eight instead of 29 B-readings. The estimation of PAI through the average value of protocol SFC and SAR showed the highest correlation, but strongly underestimated LAI (Figure 3C).

All regression lines shown in Figure 3 have significantly different slopes (determined by analysis of covariance) from the 1:1 relationship. The exclusion of certain rings of the optical sensor did not lead to a better correlation between directly measured LAI and estimated PAI. PAI measurements after leaf removal actually resulted in PAI values higher than zero for all three measuring protocols, due to the influence of posts, fruit, and branches on PAI measurements. The PAI values obtained after leaf removal accurately correspond to the intercepts of the regression equations presented.

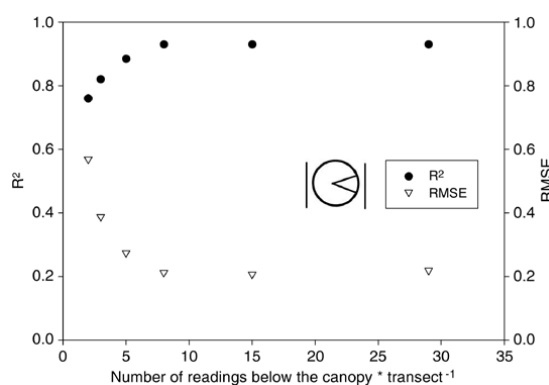


Figure 2 Coefficient of determination (R^2) and root mean square error (RMSE) dependent on the number of readings below the canopy per transect for protocol SFC (sensor facing the canopy).

The regression equation of protocol SFC (eight B-readings) can be inverted and applied as a calibration curve (Figure 4), leading to a highly significant linear relationship ($R^2 = 0.93$).

In order to assess the basis of the quality of the correlation between estimated PAI and directly measured LAI, the sensor's field of view in protocol SFC was taken into consideration. In applying the trigonometric cosine function and the Pythagorean theorem to the field of view of the PCA (Figure 5) using protocol SFC (eight B-readings), it can be deduced that for all the eight measurements within the transect the adjacent row in the direction of the view cap is taken into account. The calculation of the field of view of the outer concentric

ring (maximum zenith angle 74°) for the first B-reading of the transect is shown exemplarily for the whole transect because it is the measurement where the third adjacent row is farthest from the sensor head:

$$\begin{aligned} \text{distance sensor to canopy} &= 67 \text{ cm} \\ \text{maximum zenith angle of the fifth concentric ring} \\ \text{of the sensor head} &= 74^\circ \\ \alpha &= 90^\circ - \text{maximum zenith angle} = 16^\circ \\ c &= b/\cos(\alpha) && \text{Eq. 1} \\ a &= \sqrt{c^2 - b^2} && \text{Eq. 2} \end{aligned}$$

$$\begin{aligned} b &= 200 \text{ cm} \\ c &\approx 208.06 \text{ cm} \\ a &\approx 57.35 \text{ cm} (< 67 \text{ cm}) \end{aligned}$$

Consequently, three adjacent rows of the same treatment are needed to accurately estimate LAI with the presented method SFC using PAI measurements and to successfully implement this method in VSP-trained vineyards.

Discussion

The regression lines of the three tested measuring protocols showed that the lines obtained by eight B-readings did not differ substantially in R^2 or in RMSE from the lines obtained

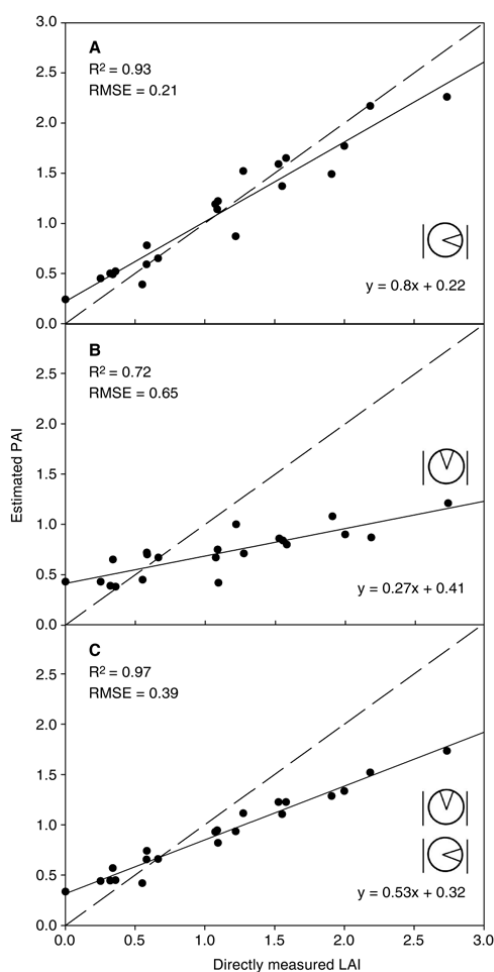


Figure 3 Comparison of two experimental protocols showing the correlation between directly measured LAI and estimated PAI for (A) protocol SFC (eight B-readings per transect); (B) protocol SAR (eight B-readings per transect), and (C) arithmetical averages of PAI of protocols SFC and SAR (eight B-readings per transect); $n = 21$. Full lines represent linear regressions and dashed lines the 1:1 relationship.

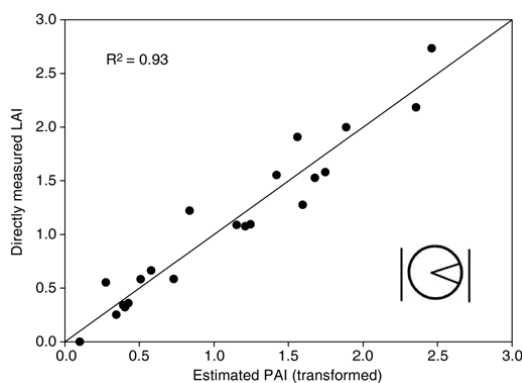


Figure 4 Correlation between estimated PAI and directly measured LAI by destructive determination using the empirical calibration equation for protocol SFC for eight B-readings ($y = 1.1684x - 0.1809$; $n = 21$).

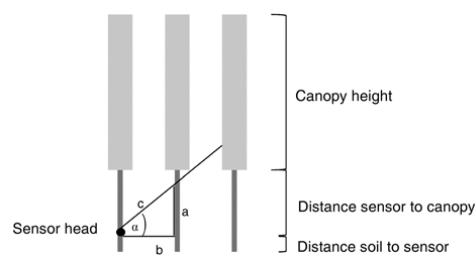


Figure 5 Cross-section of the grapevine canopy applying protocol SFC (sensor facing the canopy).

by 29 B-readings with the identical measuring protocols. This result indicates that, under narrow row width, eight readings per transect below the canopy are satisfactory for achieving accurate PAI estimates for the two different experimental protocols applied as well as for the average PAI values of the two protocols. All regression lines had significantly different slopes (determined by analysis of covariance) from the 1:1 relationship. The two tested protocols as well as the average value of the two protocols led to an overestimation of low LAI values and to an underestimation of high LAI values.

The overestimation of low LAI values is due to the influence of wooden parts and posts. Vine structure, fruit, and branches influence PAI obtained by gap fraction analysis. Therefore, the intercepts of all regression lines obtained in this study are above zero and the estimated values have to be considered PAI rather than LAI, in accordance with other studies where a contribution of wooden parts and posts to PAI was observed (Sommer and Lang 1994, Ollat et al. 1998, Patakas and Noitsakis 1999). This contribution of wooden parts and posts to light interception becomes more important when LAI is low, as the ratio of perennial parts to leaf area is higher and decreases with increasing LAI. Therefore, the PAI value obtained by measuring perennial parts without leaves should not be subtracted from the measured PAI value. Post-harvest measurements in this study showed that the intercept of the obtained regression equations accurately corresponds to the PAI values obtained after leaf removal. Other studies, in contrast, did not gain any intercept by measuring LAI in grapevine canopies (Grantz and Williams 1993, Johnson and Pierce 2004). The underestimation of higher LAI values may be due to the canopy structure of row crops such as VSP-trained grapevines. An important precondition for the use of the PCA is random distribution of foliage. The VSP grapevine canopy used in this study violated the assumption of randomly dispersed canopy elements. Aggregation of individual plants in such a row structure called clumping (Gower and Norman 1991) leads to a higher penetration of light through the canopy compared to randomly distributed elements in space and gives rise to an underestimation of LAI. This is in accordance with previous studies in discontinuous canopies (Martens et al. 1993, Sommer and Lang 1994, Patakas and Noitsakis 1999, Johnson and Pierce 2004). However, in forests where random distribution of foliage is assumed, an underestimation of LAI also was observed (Chason et al. 1991, Eschenbach and Kappen 1996), indicating that theoretical assumptions of gap fraction analysis are likely to be violated in many crops and canopies.

For a VSP-trained grapevine canopy, the application of protocol SFC is recommended because the correlation between directly measured LAI and estimated PAI is very high ($R^2 = 0.93$) and protocol SFC has the lowest RMSE of the tested methods (RMSE = 0.21) and therefore approximates the 1:1 correlation. This is important because it means that directly measured LAI does not differ substantially from estimated PAI before applying the empirical calibration equation. If canopy structure changes (unknown change that is not included in the calibration), then the error that occurs is

less important. Nevertheless, LAI was underestimated by the factor of 0.8 when protocol SFC was applied, in accordance with other findings (Patakas and Noitsakis 1999, Sommer and Lang 1994). For successful implementation of protocol SFC in VSP vineyards, at least three adjacent rows of the same treatment are needed to obtain accurate measures of PAI because the transmittance of the outermost concentric ring of the optical sensor is influenced by the third adjacent row in the direction of the view cap throughout the whole length of the transect. This finding should be taken into consideration when designing experimental field trials.

The two-azimuth-protocol, the average value of PAI of protocols SFC and SAR, led to the highest correlation coefficient ($R^2 = 0.97$), although PAI was substantially underestimated by the factor of 0.53 and therefore RMSE was quite high, consistent with previous research results (Johnson and Pierce 2004). In comparison to that study, the coefficient of determination obtained here was much higher, perhaps also due to the indirect estimation of LAI using leaf weight in the study of Johnson and Pierce (2004).

Protocol SAR did not show a high correlation between directly measured LAI and estimated PAI and RSME was high, indicating that actual LAI was strongly underestimated. This is consistent with previous findings of LAI measurement protocols in row crops along a transect viewing along the row (Ollat et al. 1998). Nevertheless, protocol SAR could be applied for canopy structure analysis using the integral of the transmission of the first four out of five concentric rings which accurately reflect the coverage of the interrow space and canopy density. This coverage is decisive for light exposure and aeration of the bunch zone, which again determine fruit quality, phenol content, and health status of the grapes. The integral of the transmission of the first four concentric rings of protocol SAR determined with the PCA could be correlated with PointQuadrat analysis in future studies (Norman and Campbell 1989, Smart and Robinson 1991).

Conclusion

The present work represents the first study of LAI estimation by gap fraction analysis with an adequate, equally distributed number of samples in a wide range of LAI for VSP-trained grapevines which shows a high coefficient of determination and takes into account the influence of wooden parts and posts. Protocol SFC (sensor facing the canopy) gave accurate estimates of LAI by measuring PAI in VSP-trained grapevines (*V. vinifera* L. cv. Riesling) along a diagonal transect including eight vines on each side using a plant canopy analyzer. If the empirical calibration equation is available, then the measurements may provide accurate LAI estimates.

Nevertheless, local calibration will be required to adapt the protocol to specific parameters such as vine and trunk height, planting density, vineyard management, and trellis system. The information provided facilitates the adaptation of the protocol to other vineyards or row crops because of the detailed description of the LAI development along the transect and minimum requirements of B-readings for accurate LAI estimation. The protocol offers a useful tool for rapid

and precise LAI estimation in VSP systems and for supporting management decisions based on LAI that influence grape quality and yield.

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Chapter 3 Soil water-holding capacity mediates hydraulic and hormonal signals of near-isohydric and near-anisohydric *Vitis* cultivars in potted grapevines²

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Soil water-holding capacity mediates hydraulic and hormonal signals of near-isohydric and near-anisohydric *Vitis* cultivars in potted grapevines

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Abstract. Grapevine (*Vitis vinifera* L.) expresses different responses to water stress, depending not only on genotype, but also on the influence of vineyard growing conditions or seasonality. Our aim was to analyse the effects on drought response of two grapevine cultivars growing on two soils, one water draining (WD) containing sand 80% volume and the other water retaining (WR), with no sand. Under these two different water-holding capacities Syrah, displaying a near-anisohydric response to water stress, and Cabernet Sauvignon (on the contrary, near-isohydric) were submitted to water stress in a pot trial. Xylem embolism contributed to plant adaptation to soil water deprivation: in both cultivars during late phases of water stress, however, in Syrah, already at moderate early stress levels. By contrast, Syrah showed a less effective stomatal control of drought than Cabernet Sauvignon. The abscisic acid (ABA) influenced tightly the stomatal conductance of Cabernet Sauvignon on both pot soils. In the near-anisohydric variety Syrah an ABA-related stomatal closure was induced in WR soil to maintain high levels of water potential, showing that a soil-related hormonal root-to-shoot signal causing stomatal closure superimposes on the putatively variety-induced anisohydric response to water stress.

Additional keywords: abscisic acid (ABA), cavitation, embolism, hydraulic conductance, water potential.

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Introduction

Grapevine (*Vitis vinifera* L.) is a species expressing both isohydric and anisohydric behaviours, depending not only on genotype (Schultz 2003), but also on the influence of growing conditions or seasonality (de Souza *et al.* 2003; Chaves *et al.* 2010) or on the environmental conditions to which the plant was exposed (Collins *et al.* 2010; Lovisolo *et al.* 2010; Pou *et al.* 2012; Tramontini *et al.* 2013a).

Although the genotype itself is not sufficient to preview the physiological behaviour of grapevine plants, some cultivars have been more frequently observed expressing consistent results than others. One of these is Syrah. This cultivar, of mesic origin, has been mainly categorised as anisohydric, either from observations of plants under field conditions (Schultz 2003; Rogiers *et al.* 2009; Soar *et al.* 2009) or in pots (Soar *et al.* 2006). Cabernet Sauvignon, in contrast, has been more frequently observed to display a response to water deprivation nearer to isohydric type (Hochberg *et al.* 2013). Owing to the differential response

observed on these two cultivars under the same water conditions, Cabernet Sauvignon and Syrah have already been coupled in comparative experiments (Chalmers 2007; Petrie and Sadras 2008; Rogiers *et al.* 2009; Hochberg *et al.* 2013) and can therefore be selected as efficient models for representing iso- and anisohydric behaviours.

Stomatal control, an endogenous but highly variable character, was considered in combination with the soil effect. Soil is another crucial component in grape and wine production, not only because it determines the water and nutrient availability for the plant and therefore its productive performances, but also for its specific implication in the 'terroir' effect in viticulture (Bodin and Morlat 2006; van Leeuwen *et al.* 2009). Despite the acknowledged soil importance on grape and wine production, not many studies have attempted to quantify its effects with comparative trials. For this reason, in the presented work, we decided to focus our attention only on the differences produced by two soils in terms of soil texture and

related water availability provided to the plant: one single aspect that is strongly influenced by physical, chemical and biological properties of the substrate.

When a soil dries the increasing drought affects the plant in multiple and complex ways (Whitmore and Whalley 2009). Cavitation of the xylem vessels is a very relevant consequence of the limited soil moisture, as it can produce dramatic consequences by reducing the hydraulic conductivity of the vascular tissues and impairing the possibility for the plant to replace transpired water (Brodersen *et al.* 2013). It is also one of the most studied effects of drought in grapevine, in combination with loss in hydraulic conductance (Lovisolo and Tramontini 2010). In leaves, cavitation and consequent embolism formation mainly affect the leaf midrib (Blackman *et al.* 2010), with a conductivity loss in grapevine petioles of 50% at Ψ_{stem} of -0.95 MPa and of more than 90% at -1.5 MPa (Zufferey *et al.* 2011). However, the entity of damage produced by cavitation and the break against its propagation are modulated by the speed and intensity of stomata reaction and by its effect on transpiration (Domec and Johnson 2012), approximating leaves to hydraulic fuses of the plant (Zufferey *et al.* 2011).

Embolism formation and repair is controlled by a likely hydraulic mediation at the leaf level (Pantin *et al.* 2013) and via chemical signals (Salleo *et al.* 2004; Lovisolo and Schubert 2006), among which abscisic acid (ABA) has a crucial role. Indeed, ABA is the hormone devoted to driving the stomatal response to drought: when the soil water potential declines, ABA acts as a messenger indicating water stress from the roots, via the xylem sap, to the guard cells in the leaves and inducing the stomata closure (Hartung *et al.* 2002), thus limiting the potential consequences of embolism formation (Chitarra *et al.* 2014). When the water availability is restored to an adequate level, the roots stop releasing the hormone and the stomata re-open. The delayed interruption of the signal, which is much more gradual than the initial release, suggests a further action of the hormone on the embolisms repair (Lovisolo *et al.* 2008; Perrone *et al.* 2012).

Furthermore, in grapevine metabolic and hydraulic behaviour have shown to be related, according to the observations recently published by Hochberg *et al.* (2013) in a study conducted on Cabernet Sauvignon and Syrah plants. In this work the more anisohydric grapevine cultivar showed higher water uptake and higher g_s than the near-isohydric cultivar.

The aim of the present work was to analyse the effect of two types of drying soil, differing in water retaining properties, on two grapevine genotypes, characterised by different ecophysiological behaviour, from the point of view of the hydraulic balance of the plant (i.e. water potential, stomatal control and embolism formation), and its hormonal (ABA) control of water losses.

Materials and methods

Plant material and growing conditions

The trial was conducted in August 2012 at Hochschule Geisenheim University (Geisenheim, Germany) on 16 *Vitis vinifera* L. plants (3 years old) of two genotypes: eight plants of 'Cabernet Sauvignon' and eight of 'Syrah'. Both were grafted on hybrids of *Vitis berlandieri* \times *Vitis riparia* ('161-49 Couderc' for 'Cabernet Sauvignon' and '420A Millardet Et De

Grasset' for 'Syrah') of comparable characteristics (Whiting 2004), especially in controlling the interrelationship between leaf or stem water potential and stomatal conductance (Tramontini *et al.* 2013b). The plants were maintained under glasshouse conditions with no supplementary light or heating in 9 L (24 cm average diameter) plastic pots filled (20 cm depth) with two different substrates, one water draining (WD soil) and the other water retaining (WR soil). The WD substrate was composed of 80% volume of sand and 20% volume of ED 73 (Einheitserde Classic, Einheitserde-Einheitserde- und Humuswerke Gebr. Patzer GmbH and Co.KG, Sinntal, Germany; consisting of 55% white peat, 30% clay, 15% sod peat; chemical properties pH (CaCl₂) 5.8, salt content 2.5 g L^{-1}) including nutrient salt ($14 + 16 + 18, 1 \text{ kg m}^{-3}$) and a slow-release fertiliser (Gepac LZD 20+10+15, 2 kg m^{-3} ; Einheitserde, Sinntal-Altengronau, Germany), the WR substrate consisted entirely of ED 73.

Plants were watered to container capacity at the beginning of the experiment (Tramontini *et al.* 2013b) and fertilised in order to bring them to the same level of nitrogen availability. Soil nitrogen content after the fertilisation was estimated according to Robinson's recommendations (Robinson 1988), confirming that at the beginning of the experiment the two different substrates had approximately the same amount of available nitrogen. Data collection started when the plants had reached a mild water stress ($\Psi_{\text{stem}} \leq -0.5$ MPa), i.e. 4 days after interruption of irrigation. In that moment plants had 14.4 ± 2.8 leaves with no significant differences between cultivars or soils. Each plant was excluded from the trial when wilting was observed.

Soil water content (θ , %), soil water potential (Ψ_{soil} , MPa), stem water potential (Ψ_{stem} , MPa), xylem embolism extent and stomatal conductance (g_s , $\text{mmol m}^{-2} \text{ s}^{-1}$) were assessed during the whole duration of the experiment. All measurements were taken daily between 0930–1200 hours and 1400–1700 hours in order to standardise putative control of circadian expression in cell water channels (Uehlein and Kaldenhoff 2006).

Water relations

Soil water content (θ) was gravimetrically determined by collecting daily ~ 10 mL of soil from three different points and depths in each pot (5, 10 and 15 cm depth with 120° of angular separation between each of the respective sample points). The soil was weighed, oven-dried at 100°C for 24 h and then reweighed to assess water content. At the same time, the water retention curves for the two soils were assessed with pressure plate measurements of the potting substrate (Richards 1965), obtaining two equations:

$$\text{WR soil} - \Psi_{\text{soil}} = 53.791 \times e^{-0.127\theta}, \quad (1)$$

$$\text{WD soil} - \Psi_{\text{soil}} = 1.3423 \times e^{-0.264\theta}. \quad (2)$$

The obtained relationships allowed for the calculation of Ψ_{soil} based on θ .

Ψ_{stem} was measured on mature, undamaged and non-senescent leaves using a pressure chamber (Soilmoisture Corp., Santa Barbara, CA, USA) (Scholander *et al.* 1965) at midday according to Turner (1988). Prior to the measurements, leaves were bagged with a plastic sheet and covered with

aluminium foil to stop transpiration at least 1 h before measurements were taken.

Xylem embolism

Daily determination of xylem embolisms in leaf petioles, induced by the presence of air bubbles in xylem vessels, was carried out around midday using a high-pressure flowmeter (HPFM, Dynamax Inc., Houston, TX, USA) (Tyree *et al.* 1995). As the assessment of embolism extent is a destructive analysis, leaf petioles were used as a proxy of the plant behaviour (Lovisolo *et al.* 2008; Perrone *et al.* 2012). During the whole duration of the experiment macro- and microbubbles were regularly flushed out of the system according to the manufacturer's instruction manual and the mismatch between the two pressure transducers was controlled daily by running the 'Set Zero' routine before measuring.

For each determination of percent loss of conductivity (PLC), the petioles and leaves were cut under water from the shoots and immediately attached to the HPFM tubing under water preventing air bubbles to enter the system. The leaves were cut ~1 cm above the petiole insertion a few seconds after starting the measurement. The initial hydraulic conductance K_{hi} was determined applying an initial pressure of ~20 kPa for 3 min. Distilled and degassed water with an addition of 10 mmol L⁻¹ KCl was used as perfusion liquid. Petioles were then flushed for 3 min applying a transient increase of pressure until a pressure of ~550 kPa was reached. This pressure was kept constant for 3 min. To determine the final hydraulic conductance K_{hf} the pressure was downregulated to ~20 kPa and held constant for 3 min. To calculate K_{hi} and K_{hf} average values of the hydraulic conductance of the respective timespans were used.

Data were displayed and stored using the software HPFM95-XP Version 1.12 (Dynamax Inc.) and exported and processed using Microsoft (Redmond, WA, USA) Excel.

The percent loss of conductivity (PLC) was determined as follows:

$$PLC [\%] = \frac{(K_{hf} - K_{hi})}{K_{hf}} * 100. \quad (3)$$

After the embolism determination the length and the maximum and minimum diameter of the petioles was assessed.

Stomatal conductance

Measurements of g_s were conducted on adult, non-senescent leaves that were well exposed to direct sunlight. Stomatal conductance was measured using a porometer (AP4, Delta-T Devices Ltd, Cambridge, UK). Measurements on three leaves per plant were taken for every measuring cycle and the g_s values of the three leaves were averaged.

Analysis of abscisic acid in leaves

ABA was extracted from leaves where stomatal conductance was assessed applying the method described by Materán *et al.* (2009) with some adaptations: 2 g of frozen tissue were grounded to powder under liquid nitrogen, 5 mL of 80% Methanol were added and the samples were extracted at 4°C overnight. Samples were centrifuged at 1500g for 5 min, the supernatant was transferred to a flask and methanol was evaporated. The pH was adjusted to

values between 8 and 9 with a phosphate buffer; 1 mL of ethyl acetate was added and samples were centrifuged at 1500g for 5 min; after discarding the supernatant, the pH was adjusted to 2–3 (with 1 N HCl), 2 mL of ethyl acetate were added and the samples were centrifuged at 1500g for 5 min. The supernatant was removed and the ethyl acetate fraction was evaporated. The dry residue was re-suspended in methanol, filtered in brown vials and injected into a 1260 Infinity HPLC-DAD System (Agilent Technologies, Cernusco sul Naviglio, Milano, Italy). ABA was separated on a Purosphere STAR RP-18, 5 µm, LiChroCART (250–4) (Merck, Darmstadt, Germany) column thermostated at 35°C. The solvent gradient used was 100% A (94.9% H₂O : 5% CH₃CN : 0.1% HCOOH) to 100% B (5% H₂O : 94.9% CH₃CN : 0.1% HCOOH) over 20 min. Solvent B was held at 100% for 10 min then the solvent returned to 100% A (Forcat *et al.* 2008). The flow rate into the column was set at 0.5 mL min⁻¹. DAD detection was performed at 262 nm, acquiring spectra in the range from 190 to 700 nm.

To quantify ABA concentration in leaf samples the external standard method was used by building a calibration curve with (±) abscisic acid, ≥98.5% (Sigma Aldrich SRL, Milan, Italy) concentration ranging from 13.5 to 54.0 mg L⁻¹. ABA identification was performed on the basis of retention times and of DAD spectrum comparison respect to the standard solution.

Statistical analysis

Regression coefficients were obtained using Excel (Microsoft, Redmond, WA, USA), and statistical analysis was performed with univariate analysis of variance (ANOVA) and multivariate analysis of variance (MANOVA) to reveal differences among cultivars and soils, by using IBM SPSS statistics 20.0 software package (SPSS, Chicago, IL). Differences between means were revealed by Tukey's test ($P < 0.05$).

Results

Interrelationships between stomatal conductance and soil and stem water potential in different soils and cultivars

Our observations excluded the initial phase of optimal water availability and focussed on the dynamics of water relations evolving from mild (day 1 of measurements) to extreme drought, as shown in Fig. 1. The soil water content between WR and WD soils was very different from the beginning; however, the dynamics of the daily averages of Ψ_{stem} and g_s did not express constant differences between soils and cultivars along the period of the trial. The proportion of embolised vessels at petiole level (PLC) was higher on WD soil than on WR for most of the trial, but not constantly along the trial.

However, the relationship between Ψ_{stem} and θ highlights how the two substrates are distinct for their effect on plant water status (Fig. 2). These differences are already evident at mild water stress conditions (Ψ_{stem} around -0.5 MPa) and when on WR soil the two cultivars show a linear relationship with Ψ_{stem} decreasing with decreasing θ (expressed as small, negative slope of regression lines), on WD the θ is so reduced that Ψ_{stem} changes substantially for any small variation of θ (expressed as higher, negative slope of regression lines).

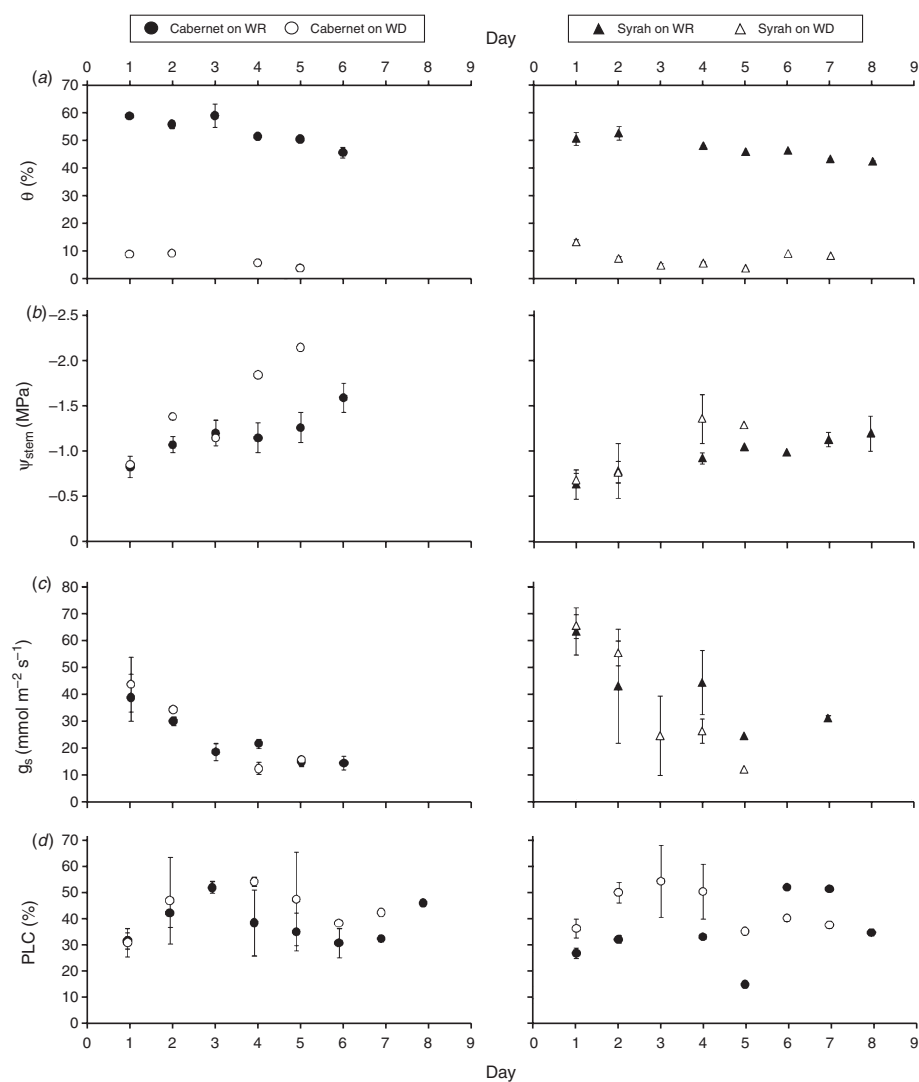


Fig. 1. (a) Dynamics of soil moisture (θ , %), (b) stem water potential (Ψ_{stem} , MPa), (c) stomatal conductance (g_s , $\text{mmol m}^{-2} \text{s}^{-1}$), and (d) percent loss of conductivity due to embolisms (PLC, %), during the days of the trial. Measurements were conducted on plants of Cabernet Sauvignon (circles) and Syrah (triangles) on water draining (WD, white) and water retaining (WR, black) soils. Means \pm s.e. Diamonds in (d) represent the mean value of the day for both cultivars grouped.

The measured Ψ_{stem} was then combined with the calculated soil water potential (Ψ_{soil}) (Fig. 3). The obtained curves show that during water stress Ψ_{stem} declined following a decrease in Ψ_{soil} . In Cabernet Sauvignon this plant adaptation was evident at mild stress conditions, and apparently delayed (or less effective) in Syrah.

The response of g_s to Ψ_{stem} was greatest at the beginning of the trial with an overlap of the two curves representing the two

cultivars at around -1.4 MPa (Fig. 4a). Compared with Syrah, Cabernet Sauvignon showed lower g_s under mild water stress conditions without strong changes under severe water stress conditions characterising its isohydric behaviour. Our experiment focused on results obtained under stress, but hypothetical relationships preceding limiting conditions can be drafted: in these conditions Cabernet Sauvignon would probably have shown a steep adaptation to water stress, whereas Syrah

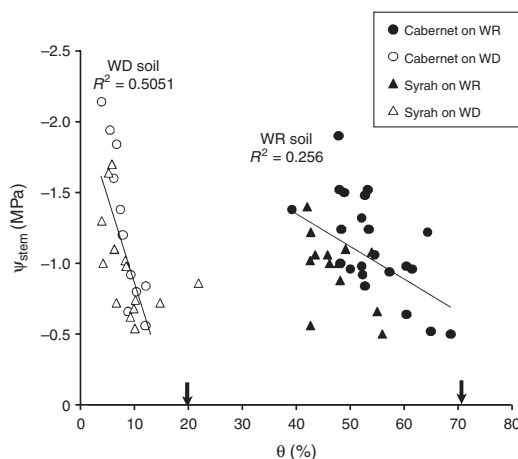


Fig. 2. Relationship between stem water potential (Ψ_{stem} , MPa) and soil moisture (θ , %) measured on plants of Cabernet Sauvignon (circles) and Syrah (triangles) on water draining (WD, white) and water retaining (WR, black) soils. Arrows on the x-axis point to maximum water-holding capacity of the two soils (% water at -0.01 MPa).

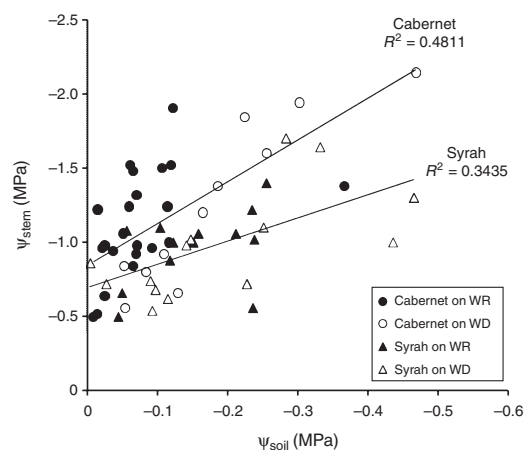


Fig. 3. Relationship between stem water potential (Ψ_{stem} , MPa) and soil water potential (Ψ_{soil} , MPa) measured on plants of Cabernet Sauvignon (circles) and Syrah (triangles) on water draining (WD, white) and water retaining (WR, black) soils. Ψ_{stem} was obtained from direct measures while Ψ_{soil} from the derived equations of Ψ_{soil} and θ .

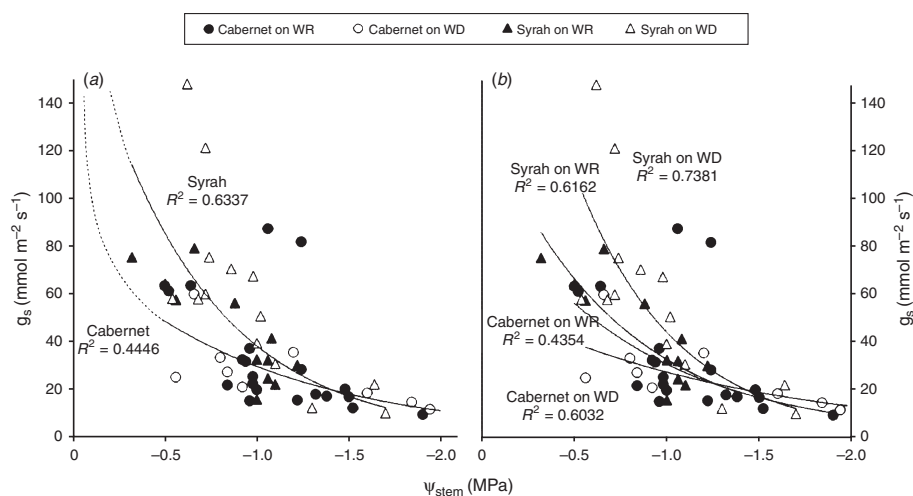


Fig. 4. Interrelationship between stomatal conductance (g_s , $\text{mmol m}^{-2} \text{s}^{-1}$) and stem water potential (Ψ_{stem} , MPa) measured on plants of Cabernet Sauvignon (circles) and Syrah (triangles) on water draining (WD, white) and water retaining (WR, black) soils. The two figures present the same data clustered only for varieties (a) and for the varieties on each soil (b). In addition, in Fig. 4a, an arbitrary hypothetical curve preceding water stress has been identified with a dashed line.

progressively coupled stomatal function with decreasing plant water status (Fig. 4a). When splitting the two curves for the soil plots, further observations can be collected (Fig. 4b). The two cultivars on WD soil maximise their differences, whereas on WR soil they become minimised. Syrah maintains generally higher g_s

values than Cabernet Sauvignon, but, although, at a given Ψ_{stem} , in Syrah g_s is higher on WD than on WR soil, the opposite occurs in Cabernet Sauvignon.

When these results are presented in form of average values, as illustrated in Fig. 5, all these differences in g_s of the two cultivars

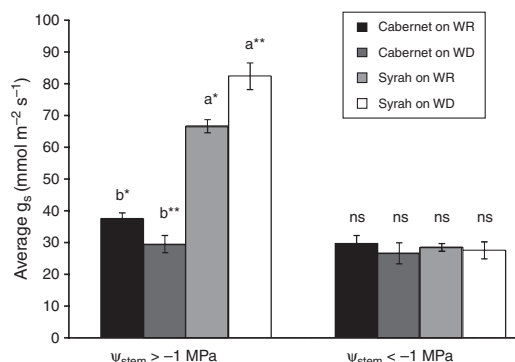


Fig. 5. Average values of leaf stomatal conductance (g_s , $\text{mmol m}^{-2} \text{s}^{-1}$) measured on plants of Cabernet Sauvignon on water retaining soil (WR, black) and on water draining soil (WD, light grey) and on Syrah plants on WR (dark grey) and on WD (white). Data have been clustered for those collected between mild and moderate water stress ($\Psi_{\text{stem}} > -1 \text{ MPa}$) and high water stress ($\Psi_{\text{stem}} < -1 \text{ MPa}$). Values of bars topped by the same letters are not significantly different; different letters indicate significantly different groups: *, $P < 0.05$; **, $P < 0.01$ (Tukey's test).

appear significantly valid at Ψ_{stem} not lower than -1 MPa , whereas no significant differences between g_s of the different cultivars occur at Ψ_{stem} lower than -1 MPa .

By sorting all measurements of stomatal conductance and stem water potential in three homogenous groups according to decreasing levels of soil water potential, it is possible to run a statistical analysis of results collected at comparable level of soil water availability (Table 1). At highest levels of soil water potential (mild water stress) the cultivar and not the soil significantly drives stomatal conductance, buffering stem water potential adjustments. When water availability in soil further decreases (intermediate water stress) soil properties significantly influence stomatal response. Under such conditions, in WR soils a stomatal closure is induced to maintain high levels of stem water potential. In Cabernet Sauvignon the putative isohydric control

on water potential is not so effective, as in parallel to a not significant stomatal closure, plants respond to water deprivation with a decrease in water potential. However, under severe water stress, stomatal control does not avoid decrease on water potential. At these severe levels of water deprivation, soil properties do not influence g_s/Ψ_{stem} response.

Embolism-related and hormone-driven plant adaptations to water stress

While observations concerning g_s are relevant for level of stress not higher than -1 MPa , the level of embolism quantified as percentage loss of hydraulic conductivity (PLC) provides relevant results also at more extreme conditions (Fig. 6). The differences observed between the two soils are statistically significant ($P < 0.05$) with the vines on WD substrates showing a significantly higher PLC than WR substrates at $\Psi_{\text{stem}} < -1 \text{ MPa}$.

The analysis of the ABA content in leaves showed that the relationship between ABA concentration and g_s was consistently dependent on soil type for Syrah but not for Cabernet Sauvignon (Fig. 7a), variety where stomatal control was tighter (Fig. 7b). In both varieties, significantly in Syrah, the WR soil induces an increase of ABA content in leaf (Fig. 7b).

Discussion

The aim of this study was to investigate how soil water-holding capacity could influence hydraulic and hormone-driven reactions of two cultivars putatively recognised as different in their stomatal response to water stress: Cabernet Sauvignon and Syrah.

Hydraulic control of water stress

Water stress effects were already apparent at mild water stress conditions (Ψ_{stem} around -0.5 MPa), when plants started to experience different shrinking capacities of the two substrates. According to Whitmore and Whalley (2009), when a shrinking soil dries, as in the WR substrate of our pots, its degree of saturation is kept small in comparison with a drying rigid soil, such as the WD soil of this experiment (Fig. 1). In WD soils, the matric potential becomes negative much faster, thus, lowering the

Table 1. Influence of cultivar and soil water-holding capacity on stem water potential (Ψ_{stem}) and stomatal conductance (g_s)

Data were divided in three classes of soil water potential (Ψ_{soil}) values: mild ($\Psi_{\text{soil}} > -0.083$), intermediate ($-0.083 > \Psi_{\text{soil}} > -0.212$) and severe water stress ($\Psi_{\text{soil}} < -0.212$), and processed separately for the two effects of cultivar and soil. Different letters indicate significant differences among means, F -test, $P < 0.05$, *post hoc* Tukey's test; n.s., not significant

| Water stress | Cultivar/Soil | Ψ_{stem} | | g_s | |
|---|---------------------------|----------------------|------|-------|------|
| Mild ($\Psi_{\text{soil}} > -0.083$) | Cabernet Sauvignon | -0.972 | n.s. | 36.1 | b |
| | Syrah | -0.764 | n.s. | 75.2 | a |
| Intermediate ($-0.083 > \Psi_{\text{soil}} > -0.212$) | Cabernet Sauvignon | -1.189 | b | 33.4 | n.s. |
| | Syrah | -0.875 | a | 55.3 | n.s. |
| Severe ($\Psi_{\text{soil}} < -0.212$) | Cabernet Sauvignon | -1.780 | b | 14.7 | b |
| | Syrah | -1.087 | a | 35.2 | a |
| Mild ($\Psi_{\text{soil}} > -0.083$) | Water retaining soil (WR) | -0.964 | n.s. | 41.9 | n.s. |
| | Water draining soil (WD) | -0.745 | n.s. | 60.9 | n.s. |
| Intermediate ($-0.083 > \Psi_{\text{soil}} > -0.212$) | Water retaining soil (WR) | -1.196 | n.s. | 27.9 | b |
| | Water draining soil (WD) | -0.867 | n.s. | 60.8 | a |
| Severe ($\Psi_{\text{soil}} < -0.212$) | Water retaining soil (WR) | -0.994 | n.s. | 19.5 | n.s. |
| | Water draining soil (WD) | -1.498 | n.s. | 22.3 | n.s. |

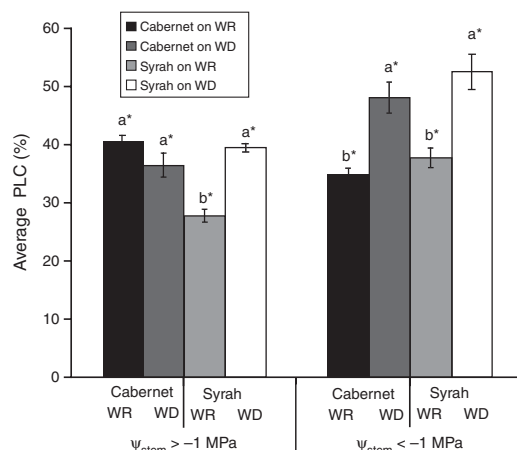


Fig. 6. Average values of percent loss of conductivity (PLC, %) due to embolism formation, measured on leaf petioles of Cabernet Sauvignon on water retaining soil (WR, black) and on water draining soil (WD, light grey) and on Syrah plants on WR (dark grey) and on WD (white). Data have been clustered for those collected between mild and moderate water stress ($\Psi_{\text{stem}} > -1$ MPa) and high water stress ($\Psi_{\text{stem}} < -1$ MPa). Values of bars topped by the same letters are not significantly different; different letters identify significantly different groups: *, $P < 0.05$; **, $P < 0.01$ (Tukey's test).

level of saturation after a much smaller amount of water is removed by roots.

In addition to the soil effect, with $\Delta\Psi$ between soil and stem higher for Cabernet Sauvignon than for Syrah, the two cultivars expressed a different capacity of water extraction from the substrate (Fig. 3), requiring to the former a higher energy in order to keep the water flow under increasing stress conditions. Furthermore and probably related to the above-mentioned reason, Syrah displays higher g_s values than Cabernet Sauvignon, especially during early phases of water stress (mild water stress) (Fig. 4). In contrast, Cabernet Sauvignon would preserve soil moisture more efficiently than Syrah, imposing at the same time a sensitive control to Ψ_{stem} while Ψ_{soil} decreases (Fig. 3). This result is consistent with putative near-anisohydric behaviour for Syrah and near-isohydric behaviour for Cabernet Sauvignon and with results recently obtained in an experiment by Hochberg *et al.* (2013). Also, a lower leaf area of the canopy could preserve soil moisture, but our pot plants were uniform and did not have different leaf area. The curves obtained from the four combinations of soil and cultivar (Fig. 4b) could be thus explained by the fact that in water-stress conditions near-anisohydric varieties do not promptly regulate their stomatal conductance and therefore, their transpiration rate (which was the case of WD substrate, Fig. 2). In contrast, near-isohydric varieties, by tightly regulating the stomatal aperture, limit more the waste of water resources. Furthermore, it can be observed how the two curves on WR substrate are closer to each other than to the respective cultivar-correspondent on WD. As already observed under field conditions (Tramontini *et al.* 2013a), the expression of plant reactions to water stress seems to be buffered on clay soils. This

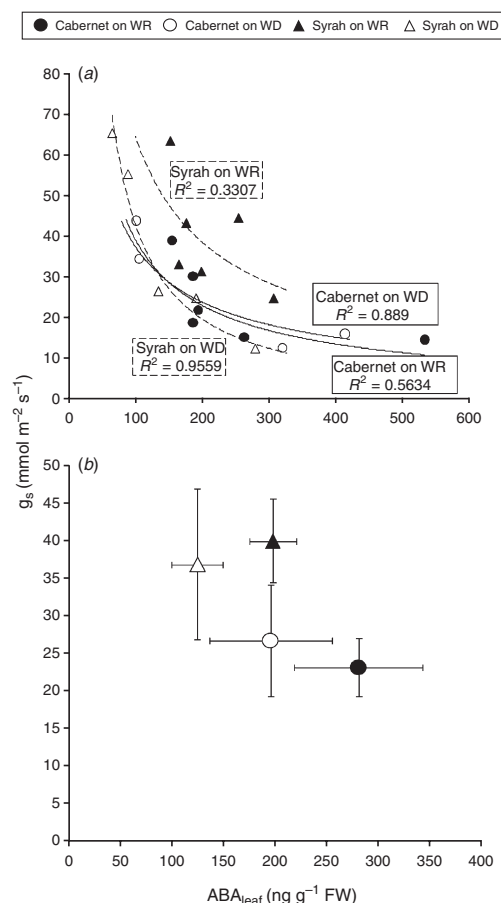


Fig. 7. (a, b) Relationship between stomatal conductance (g_s , $\text{mmol m}^{-2} \text{s}^{-1}$) and abscisic acid (ABA) concentration ($\text{ng g}^{-1} \text{FW}$) in leaf samples on plants of Cabernet Sauvignon (circles) and Syrah (triangles) on water draining (WD, white) and water retaining (WR, black) soils. In (a), continuous lines represent the two curves obtained for Cabernet Sauvignon and dashed lines for Syrah. In (b), means \pm s.e. are displayed.

could be due to the higher capacity of this kind of soils to hold water and release it gradually to the plant. It could be hypothesised that WR substrate produces an effect similar to that of clay soil, submitting the potted roots to transient drought conditions (produced by the daily fluctuations of dehydration during the day and rehydration during the night) able to interfere with the physical and hormonal signalling between roots and stem. However, as illustrated in Fig. 5, all these differences in g_s are significantly valid at Ψ_{stem} not lower than -1 MPa. When water stress becomes more severe, stomatal regulation is hydraulically controlled and a feedback on stomatal function derives from the metabolic plant control. Under increasing water stress, the

limitations to photosynthesis pass gradually from a stomatal control to a metabolic control (Flexas *et al.* 2004, 2006). Because of this, the differences between iso- and anisohydric behaviours are evident between mild and moderate water stress, where the expression of the limitations imposed at stomatal level are maximised. In our results, in these conditions, the average g_s is significantly different between varieties but not between substrates (under each variety), although on WD the differences remain evident. Concerning the consequent risk of cavitation, Syrah on both soils and Cabernet Sauvignon on WD have an increase in embolism formation, expressed in terms of xylem conductivity losses, of 32–36%, moving from $\Psi_{\text{stem}} > -1$ MPa to $\Psi_{\text{stem}} < -1$ MPa. Only Cabernet Sauvignon on WR soil shows higher embolism formation at $\Psi_{\text{stem}} > -1$ MPa than at $\Psi_{\text{stem}} < -1$ MPa. An explanation of this phenomenon would require the support of further data concerning, for example, the implication of the chemical signalling (in particular ABA) in the transpiration control. Soar *et al.* (2006) have, in fact, demonstrated the contribution of ABA to the differential response of g_s in iso- and anisohydric cultivars.

Absciscic-acid control on stomatal conductance

In the near-isohydric cultivar, Cabernet Sauvignon, expressing very similar level of cavitation on the two soils at $\Psi_{\text{stem}} > -1$ MPa, we observed a more stable ABA signal, independently from the soil (Fig. 7), similarly to observations by Puértolas *et al.* (2013) using *Phaseolus vulgaris*. In contrast, in Syrah, showing two levels of cavitation on the two soils both at moderate and at higher stress level, also the curves of ABA concentration in leaves were clearly distinguished; in fact the leaves of plants on WR soil showed higher hormone concentration than those on WD soil, showing a substrate-dependant ABA concentration, as observed by Dodd *et al.* (2010) on *Helianthus annuus*. In order to better analyse this result we suggest comparing it with that in Fig. 4b: contrary to initial expectations, Syrah has generally higher g_s on WD than on WR soil, and this may be due to the specific circumstances produced by the WR soil, as above-mentioned, favouring the release of the hormone (ABA) in the leaf. As recently observed by Brodribb and McAdam (2013) on two conifer species, the isohydric stomatal regulation can be identified as an ABA-driven stomatal closure, whereas the anisohydric is, at least initially, water potential-driven. The same appears to be true on our two grapevine cultivars: ABA control on g_s is tight in Cabernet Sauvignon and it is independent to soil properties. In Syrah plants potted on WD soil a similar ABA control on stomatal conductance subsists. However, when the anisohydric Syrah grows onto the WR soil, an additional ABA leaf biosynthesis or accumulation is recordable. The WR-induced raise in ABA allows stomatal control limiting the anisohydric response, as it happens when anisohydric grapevines are deficit-irrigated upon partial root zone drying (Stoll *et al.* 2000; Romero *et al.* 2012).

Hints for future research and speculations

Our results are in line with those recently presented by Hochberg *et al.* (2013) for a similar work conducted on the same two varieties and with the general consideration on the differential

photoprotective response to stress in iso- and anisohydric cultivars (Pou *et al.* 2012). We would expect that plant productivity of Cabernet Sauvignon would be influenced by the soil characteristics less than Syrah, due to the ABA-driven stomatal closure and its putatively stronger downregulation of photosynthesis.

The results of our current study combined with the ecological and oenological characteristics of the two genotypes, seem to find coherence: Cabernet Sauvignon, the more isohydric variety, due to a tight stomatal control, conserves varietal characteristics on the grape independently from the growing conditions. From a viticultural point of view, the avoidance of extreme conditions (and of the consequent recovery phases) to which Syrah is more prone, allows this variety to buffer vintage differences. Hence, the more anisohydric variety seems to base its stomatal control more on hydraulic signals. This could be hypothesised as the effect of a higher involvement of long-term adaptation mechanisms, such as anatomic modifications, and the development of a product which strongly varies according to the characteristics of the substrate. Both are expressions of the ‘terroir’ concept favouring different components and mechanisms to adapt.

Although our results have been obtained from potted plants, where the nature of the substrate and the available volume for root development are a limiting projection of the edaphic condition of a vineyard, nevertheless they could be of support in the interpretation of ‘terroir’ expression previously introduced by the same authors (Tramontini *et al.* 2013a). The isohydric Cabernet Sauvignon can adapt to a variety of climates and soils and, in spite of that, maintain certain organoleptic traits in the final product. It is considered extremely capable to express the characteristics of a given ‘terroir’ and, due to that, has been for a long time the world’s most widely planted premium red wine grape (Robinson 2006). The anisohydric Syrah, on the other hand, is a very common commercial variety (the world’s seventh most grown grape in 2004, still according to Robinson 2006) particularly distributed in warmer regions, from which very diverse wines can be produced.

Furthermore, ABA plays a key role by stimulating the activation of the flavonoid and anthocyanin biosynthesis pathway (Davies and Böttcher 2009; Ferrandino and Lovisolo 2014). Both its impact on water relations and on berry metabolism may contribute to a differential berry quality. This hypothesis could represent a relevant topic for further studies in field conditions, where also long-terms mechanisms of adaptation and more complex dynamics of hormonal signalling (Dodd 2013) can be observed, and extended to other varieties, considering the main mechanisms involved in the ‘terroir’ expression.

Conclusions

In conclusion, we reported a hydraulic control of stomatal responses at the base of the near-anisohydric Syrah adaptations to water stress, in contrast to an ABA-induced stomatal control in the near-isohydric Cabernet Sauvignon. Also in Syrah, however, the hormone-related response could be effective when soil properties allowed for higher water storage buffering hydraulic adaptations.

Conflicts of interest

The positions and opinions presented in this article are those of the author (primary) alone and are not intended to represent the views or scientific works of EFSA.

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Chapter 4 Growth, Yield and Fruit Quality of Grapevines under Organic and Biodynamic Management³

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RESEARCH ARTICLE

Growth, Yield and Fruit Quality of Grapevines under Organic and Biodynamic Management

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Abstract

The main objective of this study was to determine growth, yield and fruit quality of grapevines under organic and biodynamic management in relation to integrated viticultural practices. Furthermore, the mechanisms for the observed changes in growth, yield and fruit quality were investigated by determining nutrient status, physiological performance of the plants and disease incidence on bunches in three consecutive growing seasons. A field trial (*Vitis vinifera* L. cv. Riesling) was set up at Hochschule Geisenheim University, Germany. The integrated treatment was managed according to the *code of good practice*. Organic and biodynamic plots were managed according to Regulation (EC) No 834/2007 and Regulation (EC) No 889/2008 and according to ECOVIN- and Demeter-Standards, respectively. The growth and yield of the grapevines differed strongly among the different management systems, whereas fruit quality was not affected by the management system. The organic and the biodynamic treatments showed significantly lower growth and yield in comparison to the integrated treatment. The physiological performance was significantly lower in the organic and the biodynamic systems, which may account for differences in growth and cluster weight and might therefore induce lower yields of the respective treatments. Soil management and fertilization strategy could be responsible factors for these changes. Yields of the organic and the biodynamic treatments partially decreased due to higher disease incidence of downy mildew. The organic and the biodynamic plant protection strategies that exclude the use of synthetic fungicides are likely to induce higher disease incidence and might partially account for differences in the nutrient status of vines under organic and biodynamic management. Use of the biodynamic preparations had little influence on vine growth and yield. Due to the investigation of important parameters that induce changes especially in growth and yield of grapevines under organic and biodynamic management the study can potentially provide guidance for defining more effective farming systems.

Introduction

The negative impact of agriculture on the environment has increased since agricultural production intensified [1,2]. Organic farming systems with their holistic approach can be seen as a

possibility to face these problems and to minimize the negative impact of agriculture on the environment [3]. “Organic agriculture is a holistic production management system which promotes and enhances agro-ecosystem health, including biodiversity, biological cycles, and soil biological activity. It emphasizes the use of management practices in preference to the use of off-farm inputs [. . .]. This is accomplished by using, where possible, agronomic, biological, and mechanical methods, as opposed to using synthetic materials, to fulfill any specific function within the system” [4]. Organic agricultural practice has to be adapted to local farming, climatic, geographical as well as social factors [3]. Research on organic farming can help adapt the system to these local factors and can furthermore investigate the effects of the production system on the ecosystem, soil, plants, food quality and economic performance under different conditions and therefore help to improve the production systems.

Demand and production of organic crops have been growing exponentially in the last few decades around the world [5,6]. Perennial crops account for about 3.2 million hectares of agricultural land worldwide. With almost nine percent, perennial cropland has a higher share in organic agriculture compared to total agriculture. Together with coffee and olives, grapes are among the most important perennial crops [7]. In most winegrowing countries organic viticulture is gaining more and more importance, but in most non-European countries organic viticulture is still in the initial stages [8]. The organically managed viticultural area in Europe increased substantially from 43000 ha in 1998 to 230000 ha in 2011, corresponding to around 5.3% of all vineyards [7,9]. Worldwide, 2.3% of all vineyards are managed according to organic standards. Furthermore, some of the world’s most prestigious wine producers have converted to organic and biodynamic viticulture [10]. This might be one reason for the increased interest in these management systems from both consumers and producers.

Research on organic versus conventional farming

Comparisons of organic and conventional farming have long been a common topic and a great deal of knowledge on organic agriculture has been accumulated. Many studies concentrated on soil quality, yield, economic performance and environmental impact, among them several long-term field trials. Organically farmed soil had significantly higher soil organic matter content [11–13], less soil erosion, larger topsoil depth [11], showed increased biological activity [13–15], lower bulk density [14–16], and higher soil quality [17,18] for various crops. The organic system showed higher soil nitrogen content [13] and reduced carbon and nitrogen losses [12], but showed lower phosphorus levels compared to conventional treatments under Australian conditions [19]. Yield under organic management decreased from 14 up to 67% compared to conventional agriculture for many crops such as potatoes, winter wheat, grass-clover [20], grain, sunflower, common wheat, sugar beet [21], cotton [22], soybean [23] and maize during conversion [13]. Other studies did not detect significant differences in soybean yields [22], maize yields [12,13] and pigeon bean yields [21] between organic and conventional production. Organically grown pears, peaches and apples did not differ in yield from conventionally produced fruit [16,17,24]. Concerning the environmental impact, the organic systems showed efficient resource utilization as well as enhanced floral and faunal diversity [20] and maintained soil productivity [11,12]. Organic cropping systems are therefore considered more sustainable than conventional cropping systems from an environmental standpoint.

Lately, a lot of research has been done on food quality of vegetables and fruit, among them several perennial crops. Organically grown tomatoes had smaller fruit size and mass, but were of better quality, had higher soluble solids, higher vitamin C content [25] and a significantly higher amount of flavonoids compared to conventionally produced tomatoes [26]. This might be due to increased oxidative stress during fruit development [25]. Organically produced

strawberries were of higher quality [18]. Organically produced pears did not vary significantly in storage life, fruit weight, pH and soluble solids from conventional pears [24], but organically produced apples were sweeter and less tart than conventional apples [17].

Research on the biodynamic farming system

Investigations on biodynamic farming systems are scarce in contrast to organic farming systems, which attracted considerable interest in the scientific community. The biodynamic agricultural movement started in the 1920s and it has been further developed in the following decades and has been institutionalized by the international certification label DEMETER. Biodynamic farming can be regarded as a form of organic agriculture. In addition to methods in organic cropping, biodynamic farming emphasizes biodiversity, influence of celestial bodies and the concept of the farm as an organism. Furthermore, a series of fermented manure, plant, and mineral preparations (divided into field spray and compost preparations) are applied on soil, crops, and compost [27]. These preparations are claimed to stimulate soil nutrient cycling and compost development and to promote photosynthesis. While the biodynamic farming system is recognized as an organic cropping system and its advantages as such are undoubted, the effects of the biodynamic preparations are still unconfirmed.

Research on biodynamic farming revealed a behavior similar to the organic farming system concerning soil characteristics, yield and growth of agricultural crops and economic performance, resource utilization and biodiversity [19,20,22,28–32]. Some authors report an increase of storage life of crops under biodynamic production [29,32] or minor differences in product quality [19,20,32], but results are not consistent. That is why it is still controversial whether biodynamic preparations as such have any effects or benefits [28–30,32–48].

Research on organic and biodynamic viticulture

In viticulture, few studies exist concerning the influence of organic management on growth, yield and grape or wine quality. The number of scientific studies investigating biodynamic viticulture is even more restricted. The major effects of organic compared to integrated or conventional viticulture are increased soil microbiological activity [49,50], increased soil organic carbon [49,51], decreased growth expressed as reduced pruning weight and reduced shoot length [52–55] as well as decreased yields [52–57]. In some cases reduced berry weight [55,57,58] and reduced number of berries per cluster [53], increased disease frequency of *Botrytis cinerea* (Botrytis) [56] and increased production costs [56,58–60] were observed in organic viticulture. Grape composition, wine quality and wine sensory characteristics are less influenced by the management regime [52,54,56,58,61–64]. Biodynamic viticulture showed reduced yields [56], a reduced ratio of yield:pruning weight [10] and reduced disease frequency of Botrytis [56] compared to organic viticulture. In a recent study, red wines from biodynamic production showed decreased alcohol content, decreased phenolic compounds, decreased wine color, decreased total polymeric pigments and decreased tannin concentration [65]. Soil quality [10], macronutrient supply in leaves [55,58], grape composition [10,56,63,64] and wine sensory characteristics [56,65] do not seem to be affected by biodynamic practices in comparison to organic viticulture.

However, there is a lack of research on the underlying mechanisms that induce changes in organically grown perennial crops [66]. Effects of consecutive years may overlap as [55] a consequence of the perennial growth habit of perennial crops. This makes the cause-effect relationship more complex. It might also explain the scarcity of studies dealing with the key factors responsible for the changes observed under the different management practices. The characterization of physiological processes of plants under different management systems can be helpful

to understand the mechanisms that cause the changes. This is necessary to improve agricultural practices and to determine effective farming systems. Moreover, the effect of organic agriculture on food quality is still controversial and it is still unconfirmed whether organic agriculture has any beneficial effects on the product quality [3].

The aim of this study was to compare different management systems for vineyards including integrated, organic and biodynamic production according to the latest standards of the respective production systems in viticulture. Growth, yield and winegrape quality were determined for the different vineyard management systems over three consecutive seasons from 2010–2012. Beyond that, general principles responsible for the various effects of the different management systems were investigated. This included the detection of nutrient status, physiological performance and disease incidence. The study can potentially contribute to a better understanding of long-term effects of organic farming on growth, yield and fruit quality of grapevines. This knowledge is crucial to improve the respective management systems and to further develop sustainable cropping systems.

Materials and Methods

Experimental site

The field experiment was conducted in Geisenheim (49° 59'; 7° 56'). The experimental site was 0.8 hectare in size and planted in 1991 (*Vitis vinifera* L. cv. Riesling, clone Gm 198–30, grafted on *Vitis berlandieri* Planch. x *Vitis riparia* Michx. cv. SO4 and *Vitis riparia* Michx. x *Vitis cinerea* Engelm. cv. Börner rootstock, respectively). The experimental site is owned by Hochschule Geisenheim University.

The vines were planted at a spacing of 1.2 m within rows and 2 m between rows using a vertical shoot positioning system (VSP). Until the end of 2005 the vineyard was managed according to the code of *good practice* [67]. Conversion to organic and biodynamic viticulture started in 2006.

The experiment was set up as a complete block design, where the three factor levels of the main effect management system were replicated in four blocks. Each main plot for the factor management system was subdivided into two subplots, which were used for the two levels of the main effect rootstock. Each plot consisted of four rows with 32 vines each. Only the inner two rows of each plot were used for data collection. The outer rows were considered as buffer rows.

The plots were checked for uniformity prior to data collection using a balanced fixed factorial analysis of variance (with factors treatment, block) with respect to particle size distribution, soil moisture, pH, humus content, C/N ratio, and phosphorus, magnesium and potassium content. Treatments did not differ significantly in any of these parameters (S1 Table).

Grape clusters of the respective treatments were analyzed for residues of systemic plant protection agents in 2009 to determine the impact of close neighborhood of integrated and organic plots on residue levels [68]. Active agents were investigated on clusters by GS-MS in Landesbetrieb Hessisches Landeslabor using an official protocol for residue detection [69]. No residues of systemic plant protection agents used in the integrated pest management could be found in the organic plots adjacent to the integrated plots (S2 Table). Therefore the plot size was considered suitable for detecting effects of the respective management system. The level of active agents found on clusters from integrated plots were below the maximum residue level (S2 Table) [70].

A weather station located approximately 500 m from the trial site was used for climate data collection. Data of weather conditions during the three seasons 2010 to 2012 are provided in S1 Fig. Long term annual rainfall for the site is 540 mm [71]. Total rainfall in the three seasons

2010–2012 was 659 mm, 469 mm and 531 mm, respectively. Growing season rainfall was 426 mm, 306 mm and 330 mm for the seasons 2010–2012, respectively.

Management

The integrated treatment was managed according to the *code of good practice* [67]. Organic and biodynamic plots were managed according to Regulation (EC) No 834/2007 [72] and Regulation (EC) No 889/2008 [73] and according to ECOVIN- and Demeter-Standards, respectively.

All three treatments received compost during the period of conversion. After analysis of the composts the same amount of nitrogen equivalents were applied to every treatment. Green waste compost was used for the integrated plots and farmyard manure for the organic and biodynamic plots. In addition, biodynamic compost preparations 502–507 were applied to the compost for the biodynamic plots.

Both, organic and biodynamic treatments received identical soil and vine management practices except that biodynamic preparations were only applied to the biodynamic plots. The Wolff-Mixture[®] was used as cover crop (S3 Table) in both, the organic and biodynamic plots. Nitrogen supply of the organic and the biodynamic treatment was ensured by breaking up and tilling under the cover crop mixture (rich in legumes) of every second row shortly before full-bloom. In the integrated plots a grass mixture was established as cover-crop in between the rows. Every second row was ploughed shortly before bloom together with the cover crop of the organic and the biodynamic treatments. The integrated plots are amended with mineral fertilizers exclusively (50 kg N*ha⁻¹*a⁻¹ on 06/26/10, one day after full-bloom and 25 kg N*ha⁻¹*a⁻¹ on 07/05/12, six days after full-bloom) to compensate for the nitrogen introduction in the organic and the biodynamic treatment that occurred due to the ploughing of the cover crop rich in legumes.

In the organic and the biodynamic treatments mechanical under-vine management was implemented. In the integrated plots weeds in between the vines were controlled by herbicides.

Erysiphe necator and *Plasmopara viticola* (powdery and downy mildew) were controlled by applying systemic fungicides in integrated viticulture. Bitter salts MgSO₄ were applied in the integrated treatment on 08//13/10, 07/11/11 and 07/26/11 and magnesium nitrate fertilizer was applied on 08/02/12 and 08/14/12. Botryticides were applied twice a year (S4 Table). For disease control in the organic and the biodynamic treatments copper, sulfur, and plant strengtheners (Mycosin VIN[®], sodium bicarbonate, sodium silicate) were used to control powdery and downy mildew (S5 Table). In all treatments RAK[®] 1+2 M (500 dispensers*ha⁻¹; 178 mg of (E, Z)-7,9-Dodecadienylacetate per dispenser and 205 mg of (Z)-9-Dodecenylacetate per dispenser) was applied against the vine moth and the European grapevine moth (*Eupoecilia ambiguella* and *Lobesia botrana*) following the mating disruption method.

The biodynamic field spray preparations horn manure and horn silica were each applied three times a year. Horn manure was applied once after harvest and twice in spring and horn silica was applied at grapevine phenological stages shortly before full-bloom, at veraison and shortly before harvest. In case no compost was applied to the biodynamic plots, the cow pat pit preparation was applied once a year in the growing season in parallel with tillage.

An overview of the management of the different treatments is given in Table 1.

Growth

Phenological stages were determined according to Coombe [74]. For this purpose 15 organs, i.e. buds, shoots or bunches per row were taken into account. Lateral leaf area was measured non-destructively at veraison. In 2010 the model of Lopes and Pinto [75] was applied, in 2011 and in 2012 the model of Mabrouk and Carbonneau [76] was applied. Both models have been

Table 1. Overview of the management of the different management systems in this study.

| Management practice | biodynamic | organic | integrated |
|-------------------------|--|-----------------------------------|-------------------------------|
| cover crop | Wolff-mixture | | grass mixture |
| under-vine-management | mechanically | | herbicides |
| fertilization | ploughing up cover crop + compost with biodynamic preparations | ploughing up cover crop + compost | mineral fertilizers + compost |
| plant protection | copper + sulfur + plant strengtheners | | systemic fungicides |
| biodynamic preparations | horn manure, horn silica, compost preparations | - | - |

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shown to be applicable for estimating lateral leaf area of *Vitis vinifera* cv. Riesling under different management systems [77]. The calibration equations adapted to *Vitis vinifera* cv. Riesling were used for the respective models. Lateral leaf area of 6 shoots (2010) and 9 shoots (2011 and 2012) per row was determined on 2 and 3 vines, respectively, measuring lateral leaf area of one primary shoot at the beginning of the cane, one in the middle and one at the end of the cane. Whole-plant lateral leaf area was obtained by multiplying the secondary leaf area per shoot with the average number of shoots per vine. Leaf area index (LAI) was estimated in 2012 using the Plant Canopy Analyzer (PCA, LAI-2200, LI-COR, Lincoln, NE, USA) as described by Döring et al. [78]. Four measurements per treatment were carried out on 09/05/12, one in each block comprising eight vines on each side of two adjacent rows. Pruning weight of every vine of the central rows was determined gravimetrically in all three growing seasons. Relative levels of total chlorophyll in leaves were estimated at full-bloom, veraison and before harvest in the three growing seasons 2010 to 2012 using a portable chlorophyll meter (SPAD-502, KONICA MINOLTA INC., Tokyo, Japan). SPAD values are highly correlated to Chlorophyll content [79,80]. Nine mature, non-senescent leaves per row with comparable plastochron indices [81] were measured and three measurements per leaf were done (base, middle, leaf tip).

Nutrient Status

Mineralized soil nitrogen content (N_{min}) was measured at the phenological stages of full-bloom, pea-sized berries and shortly before harvest. Four samples per row in two depths (0–30 cm and 30–60 cm, respectively) were taken with a soil core sampler. Two rows per management system in each plot were sampled and analyzed separately. Samples were homogenized with a soil homogenizer (Schäfer, Euskirchen, Germany). Samples were analyzed according to Schaller [82] by flow injection analysis at 540 nm using a FOSS Tecator FIAstar Analyzer (FOSS, Hillerød, Denmark).

Nitrogen, phosphorus, magnesium and potassium content in grapevine tissue were measured at full-bloom and veraison during the three seasons, as recommended by Robinson [Robinson 2006]. For this purpose ten healthy leaves per row opposite to the first inflorescence or the first cluster of a shoot were picked. The leaf blade was washed with distilled water, dried at 60°C, ground to a fine powder by Foss Cyclotec™ 1093 (FOSS, Hillerød, Denmark). 0.25 g of the ground leaf tissue was used for the wet decomposition procedure. The samples were digested for 1 ½ hours at 100°C with 10 mL of a mixture of 420 mL H₂SO₄ conc., 330 mL 30% H₂O₂, 0.48 g selenium and 14 g Li₂SO₄ according to Schaller [82]. Samples were analyzed by inductively coupled plasma with optical emission spectroscopy (ICP-OES, Spectro Arcos, Spectro Analytical Instruments GmbH, Kleve, Germany). Standard curves were obtained using a multi-element standard solution Multielement-Standardlösung “Stammlösung Blatt” 8 Elemente in Salpetersäure 1 mol·L⁻¹ (Bernd Kraft GmbH, Duisburg, Germany). Individual

readings are the average of two measurements and varied by less than 5%. Nitrogen in the leaf tissue was analyzed by flow injection analysis using a FOSS Tecator FIAstar 5000 Analyzer (FOSS, Hillerød, Denmark).

Physiological Performance

Leaf gas exchange measurements [net assimilation (A) and transpiration rate (E)] and stomatal conductance measurements [g_s] were carried out on mature, non-senescent leaves with comparable leaf plastochron indices [81] on sunny days between 9 to 12 am. The leaves selected were well-exposed to direct sunlight ($PAR > 1000 \mu\text{mol m}^{-2}\text{s}^{-1}$). Gas exchange was measured using an open gas exchange system (GFS 3000, Walz, Effeltrich, Germany). Pre-dawn water potential [Ψ_{pd}] was determined in 2011 and 2012 on mature, undamaged and non-senescent leaves using a pressure chamber [83] (Soilmoisture Corp., Santa Barbara, CA, USA) according to Turner [84]. Prior to the measurements leaves were wrapped in polyethylene bags and detached from the shoot with a single cut.

Yield

Crop yield was determined gravimetrically at harvest on 10/13/10, 09/20/11 and 10/10/12, respectively, on all vines in the plot except the buffer rows. Leaf area to fruit weight ratio [85] was determined in 2012 using LAI-measurements for leaf area estimation of the whole canopy and crop yield, as described above. Cluster weight [g], cluster length [cm] and cluster compactness index [g cm^{-2}] were determined before veraison in 2012. Three healthy clusters per row (first clusters) were randomly selected and analyzed for cluster weight and cluster length. Cluster compactness was calculated as the ratio of cluster weight [g] to cluster length squared [cm^2] according to Tello and Ibáñez [86].

The percentage of yield difference in the organic and the biodynamic treatments compared to the integrated management was calculated. The influence of berry weight, cluster weight and disease incidence and severity of downy mildew on yield reduction was estimated. Data of average single berry weight shortly before harvest, disease frequency of downy mildew before veraison and cluster weight at veraison in 2012 were used to estimate the influence of these parameters on yield reduction in the organic and the biodynamic treatments.

Disease Incidence and Severity

Since the infestation with downy mildew potentially decreases grapevine yield, disease incidence and severity on clusters was monitored on 07/15/10, 07/01/11 and 07/13/12, respectively, according to organization Eampp guidelines [87]. For this purpose 100 clusters per row were used for estimation of disease severity, 50 on each side of the canopy. Disease incidence and severity were rated on a scale of 1 to 7, where 1 corresponds to no disease and 7 corresponds to 75–100% disease.

Infestation with Botrytis on clusters was determined shortly before harvest on 10/08/10, 09/19/11 and 10/09/12, respectively, following the Eampp guidelines [87] mentioned above. For this purpose 100 clusters per row were used for estimation of disease severity, 50 on each side of the canopy. In parallel with the determination of infestation with Botrytis disease frequency of sour rot on clusters was detected shortly before harvest.

Winegrape Quality

Representative maturity samples (100 berries per row on each date) were collected to determine fruit quality parameters. Mean single berry weight of the samples was determined

gravimetrically. The juice of the samples was obtained by pressing the berries with a sampling press at 1 bar (Longarone 85, QS System GmbH, Norderstedt, Germany) for two minutes the day after sampling. Maturity sampling took place every two weeks after veraison in 2010 and 2011 and every week after veraison in 2012. The concentration of α -amino-acids (N-OPA) in the juice was determined according to Dukes and Butzke [88]. α -amino acid groups were derivatized with o-phthalaldehyde/N-acetyl-L-cysteine (OPA/NAC) reagent. Absorbance at 335 nm was measured with a UV/VIS spectrometer (SPECORD 500, Analytik Jena AG, Jena, Germany) against a juice blank. Results were calculated as mg isoleucine equivalent from a standard curve. The must was analysed for soluble solids ($^{\circ}$ Brix) by refractometry and for total acidity and pH by Fourier-transform infrared spectroscopy (FTIR) (FT2 Winescan, FOSS, Hillerød, Denmark).

Statistical Analysis

A balanced fixed factorial analysis of variance was carried out using the model

$$y = \mu + s_i + r_j + b_k + q_l + (sr)_{ij} + (sq)_{il} + e_{ijkl} \quad (1)$$

where

μ is the mean,

s_i ($i = 1..3$) are the effects of the management system, r_j ($j = 1,2$) are the effects of the rootstock, b_k ($k = 1..4$) are the block effects, q_l ($l = 1..3$) are the year effects, and e_{ijkl} is a random error term. The effects $(sr)_{ij}$ and $(sq)_{il}$ are interactions between the corresponding main effects.

If a main effect or an interaction was significant ($p < 0.05$), a Tukey test was carried out to compare the factor levels. Calculations were carried out with the AOV and Tukey's HSD commands of the statistical software R [89]. For all the parameters measured averages per combination of treatment:rootstock:block ($n = 1$) were calculated and used for statistical analyses. For certain parameters that vary over time such as mineralized nitrogen content N_{min} in the soil, assimilation rate A , transpiration rate E , stomatal conductance g_s , pre-dawn water potential Ψ_{pd} and berry quality parameters during ripening the date was also included as a fixed factor into the model. For the parameter mineralized nitrogen content in the soil the factors soil management (cover crop or cultivated soil) and sampling depth (0–30 cm and 30–60 cm) were included into the model as a fixed factor. For parameters measured in just one season such as leaf area index (LAI) and cluster compactness parameters, the factor year and the interactions with the factor year were removed from the model. In case of LAI, the rootstock was not taken into account because data collection equally included the rootstocks Boerner and SO4 by measuring within a transect of two adjacent rows.

Results

Growth

Lateral leaf area differed significantly among treatments (Table 2). The integrated treatment showed the highest lateral leaf area with 4.26 m² per plant and differed significantly from the other two treatments. The organic and the biodynamic management systems showed an average lateral leaf area of 3.45 m² and 2.95 m² per vine, respectively, and did not differ significantly from each other. Lateral leaf area differed significantly among years. 2012 showed a significantly lower leaf area compared to 2010 and 2011.

LAI assesses whole plant leaf area which is influenced by both main shoot and lateral leaf area. It differed significantly among treatments on 09/05/12 shortly before harvest. The integrated treatment again showed the highest LAI value of 2.44 and differed significantly from the other two treatments.

Table 2. Results of the balanced fixed factorial analysis of variance (ANOVA) and results of the Tukey's test for the fixed factor management system.

| field of interest | parameter | treatment | int (means ± se) | org (means ± se) | biodyn (means ± se) | rootstock | block | year | date | interaction rootstock | interaction treatment: year | soil management | depth |
|---------------------------|--|-----------|---------------------|---------------------|------------------------|-----------|-------|------|------|--------------------------|-----------------------------------|--------------------|-------|
| growth | lateral leaf area [m ²] per vine | *** | 4.26 ± 0.25 | a 3.45 ± 0.29 | b 2.95 ± 0.23 | b | ns | *** | - | ns | ns | | |
| | Leaf area index (LAI) in 2012 | *** | 2.44 ± 0.11 | a 1.64 ± 0.07 | b 1.72 ± 0.07 | b | - | ns | - | - | - | | |
| | pruning weight [dt * ha ⁻¹] | *** | 44.9 ± 1.77 | a 38.5 ± 0.97 | b 37.4 ± 1.38 | b | *** | *** | - | * | *** | | |
| | relative chlorophyll content full-bloom | ns | 38.9 ± 0.45 | - 38.2 ± 0.37 | - 38.2 ± 0.31 | - | *** | *** | - | ns | ** | | |
| nutrient status | relative chlorophyll content veraison | *** | 42.9 ± 0.43 | a 41.5 ± 0.42 | b 40.9 ± 0.47 | b | ** | *** | - | ns | * | | |
| | relative chlorophyll content harvest | ** | 41.6 ± 0.73 | a 40 ± 0.68 | b 40.4 ± 0.75 | b | ** | *** | - | ns | ns | | |
| | Nmin [kg*ha ⁻¹] in soil | *** | 14.37 ± 1.67 | b 21.44 ± 1.69 | a 20.78 ± 1.54 | a | - | ns | *** | *** | *** | *** | *** |
| | nitrogen content in leaves [%] full- bloom | ns | 2.8 ± 0.03 | - 2.77 ± 0.04 | - 2.75 ± 0.04 | - | ** | ns | *** | - | ns | * | |
| physiological performance | nitrogen content in leaves [%] veraison | * | 2.1 ± 0.04 | b 2.16 ± 0.04 | a 2.16 ± 0.04 | a | * | *** | *** | - | ns | * | |
| | magnesium content in leaves [%] full- bloom | ns | 0.22 ± 0.007 | - 0.22 ± 0.004 | - 0.21 ± 0.005 | - | ns | *** | *** | - | ns | ns | |
| | magnesium content in leaves [%] veraison | * | 0.23 ± 0.008 | a 0.22 ± 0.005 | ab 0.21 ± 0.007 | b | ** | *** | ns | - | ns | ns | |
| | assimilation rate A [μmol CO ₂ m ⁻² s ⁻¹] | *** | 10.3 ± 0.35 | a 8.5 ± 0.3 | b 8.4 ± 0.32 | b | ns | * | *** | *** | ns | * | |
| yield | transpiration rate E [mmol m ⁻² s ⁻¹] | *** | 2.42 ± 0.12 | a 1.98 ± 0.1 | b 1.98 ± 0.1 | b | ** | * | *** | *** | ns | ns | |
| | stomatal conductance g _s [mmol H ₂ O m ⁻² s ⁻¹] | *** | 117.84 ± 4.77 | a 92.72 ± 3.88 | b 90.27 ± 3.99 | b | ns | *** | *** | *** | ns | ns | |
| | pre-dawn water potential ψ _{pd} [MPa] | ** | -0.2 ± 0.01 | a -0.21 ± 0.01 | a -0.23 ± 0.01 | b | *** | *** | *** | *** | ns | * | |
| | yield [kg * ha ⁻¹] | *** | 6984 ± 559 | a 4276 ± 302 | b 4347 ± 287 | b | ns | ns | *** | - | ns | *** | |
| | leaf-area-to-fruit- weight-ratio [cm ² * g ⁻¹] in 2012 | ns | 25.11 ± 0.85 | - 32.41 ± 3.2 | - 32.94 ± 4.08 | - | - | ns | - | - | - | - | |
| | average single berry weight [g] | *** | see Fig 4 | a see Fig 4 | b see Fig 4 | b | *** | ns | ns | *** | ns | ns | |
| | cluster weight [g] at veraison in 2012 | ** | 122.29 ± 7.46 | a 101.94 ± 9.37 | b 91.92 ± 3.37 | b | ns | * | - | - | * | - | |
| | cluster length [cm] at veraison in 2012 | ns | 10.2 ± 0.34 | - 10.02 ± 0.47 | - 9.68 ± 0.28 | - | ns | * | - | - | ns | - | |
| | cluster compactness index [g*cm ⁻²] at veraison in 2012 | * | 1.17 ± 0.04 | a 1 ± 0.03 | b 0.99 ± 0.05 | b | ns | ns | - | - | ns | - | |

(Continued)

Table 2. (Continued)

| field of interest | parameter | treatment | int (means ± se) | org (means ± se) | biodyn (means ± se) | rootstock | block | year | date | interaction: rootstock | interaction: treatment: year | soil management | depth |
|--------------------------------|---|-----------|----------------------------|------------------------------|------------------------------|-----------|-------|------|------|---------------------------|---------------------------------|--------------------|-------|
| disease incidence and severity | disease incidence/severity <i>Plasmopara viticola</i> | *** | 1.02 ± 0.01 | b 2.02 ± 0.17 | a 1.89 ± 0.15 | a | ns | *** | - | ns | *** | | |
| | disease incidence/severity <i>Bortrytis cinerea</i> | ** | 4.49 ± 0.39 | b 4.64 ± 0.41 | ab 4.82 ± 0.42 | a | ns | *** | *** | - | ns | * | |
| | disease incidence/sour rot [% infested clusters] | *** | 6.17 ± 1.42 | a 0.83 ± 0.29 | b 0.62 ± 0.29 | b | ns | ns | *** | - | ns | *** | |
| winegrape quality | total soluble solids [°Brix] | ns | see S2 Fig | - see S2 Fig | - see S2 Fig | - | ns | ns | *** | ns | ns | | |
| | total acidity [g L ⁻¹] | ns | - | - | - | - | ns | ns | *** | ns | ns | | |
| | pH | ns | - | - | - | - | ns | ns | *** | ns | ns | | |
| | N-OPA | ** | see Fig 4 | b see Fig 4 | ab see Fig 4 | a | * | ** | *** | *** | ns | *** | |

*, ** and *** indicate statistical significance ($p < 0.05$; $p < 0.01$ and $p < 0.001$) of the main effects determined by ANOVA (ns = not significant). Different letters indicate statistically significant differences ($p < 0.05$) for the fixed factor management system determined by the Tukey's test (int = integrated treatment, org = organic treatment, biodyn = biodynamic treatment).

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Another important parameter for vigor is the pruning weight. The integrated treatment showed a significantly higher pruning weight compared to the organic and the biodynamic treatments (Fig 1). Average pruning weight for the integrated management system was 44.9 dt ha⁻¹, while the organic and the biodynamic treatments showed 38.5 dt ha⁻¹ and 37.4 dt ha⁻¹, respectively. The rootstock, block, and year had a significant effect on pruning weight (Table 2). Interactions between treatment and rootstock occurred. Boerner showed lower pruning weight compared to SO4 except for the biodynamic treatment in 2012. Interactions between treatment and year occurred, because the biodynamic treatment showed the lowest pruning weight except for 2012 where the organic management system showed the lowest value (Fig 1).

Relative levels of total chlorophyll index did not differ significantly among treatments at full-bloom, but later in the season it differed significantly among treatments. The two biological systems showed significantly lower chlorophyll index compared to the integrated treatment at veraison and harvest, respectively (Table 2). Interactions between treatment and year occurred concerning relative levels of total chlorophyll index at veraison. The integrated treatment showed the highest values except for 2012 where the organic plots showed the highest relative levels of total chlorophyll. The biodynamic plots showed the lowest relative levels of total chlorophyll except for 2010 where the organic treatments showed lower levels.

Nutrient Status

The organic and the biodynamic treatments showed a significantly higher mineralized nitrogen content in the soil compared to the integrated management system. The integrated treatment was fertilized with mineral fertilizers exclusively from 2010 to 2012 to compensate for the nitrate introduction by the cover crop used in the organic and the biodynamic plots. The organic and the biodynamic treatments both showed average nitrogen levels of 20 kg ha⁻¹, whereas the integrated treatment showed just an average nitrogen level in the soil of 14 kg ha⁻¹. The organic and biodynamic treatments did not differ significantly in the content of mineralized nitrogen during the growing seasons 2010 to 2012 (Table 2). The year, the date, the sampling depth and the tillage system significantly influenced the nitrate content in the soil. 2012 showed a significantly higher content of mineralized nitrogen in the soil compared to 2010 and

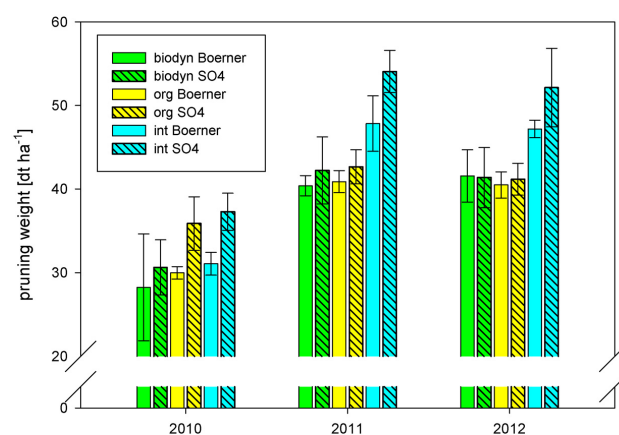


Fig 1. Pruning weight [dt ha⁻¹] from 2010–2012. Means ± sd per management system, year and rootstock (int = integrated treatment, org = organic treatment, biodyn = biodynamic treatment).

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2011, respectively. The tilled rows showed higher nitrogen content compared to the rows where cover crop was established during the growing season. In the upper soil layer (0–30 cm) there was significantly more mineralized nitrogen present than in the lower layer (30–60 cm). Interactions between treatment and year were observed. The organic treatment showed the highest levels of mineralized nitrogen in the soil except for the season 2012 where the biodynamic management system showed the highest levels.

Nitrogen and magnesium content in leaves did not differ significantly among treatments at full-bloom.

In contrast, nitrogen and magnesium content differed significantly among treatments at veraison in all three growing seasons (Table 2). The integrated treatment showed significantly lower nitrogen content in leaves at veraison, but an interaction between treatment and growing season was observed. The organic treatment showed the highest nitrogen content in the leaf tissue in 2010, whereas the biodynamic treatment showed the highest values of nitrogen in the leaf tissue in 2012. In the dry season 2011 nitrogen contents in the leaf tissue of all treatments were similar. When compared to the biodynamic system, the integrated treatment showed significantly higher magnesium content in leaves at veraison.

Physiological Performance

Assimilation rate A , transpiration rate E and stomatal conductance g_s differed significantly among treatments in the three growing seasons 2010 to 2012. Organic and biodynamic treatments showed significantly lower assimilation rate, transpiration rate and stomatal conductance compared to the integrated treatment. The mentioned parameters also differed significantly among years, blocks, and dates. The transpiration rate differed significantly between rootstocks. Boerner showed a significantly higher transpiration rate in comparison to SO4 for all treatments. An interaction between treatment and year occurred in the case of assimilation rate. The biodynamic treatment showed higher assimilation rates than the organic treatment in 2011, but showed lower assimilation rate A than the organic treatment in 2011 and in 2012 (Table 2). The development of the transpiration rate E during the growing season 2011 is shown in Fig 2. The differences in transpiration rate E among the treatments were the highest after full-bloom.

Pre-dawn water potential (Ψ_{pd}) was measured in 2011 and 2012. It significantly differed among treatments (Table 2). The biodynamic treatment showed a significantly higher level of water stress (lower pre-dawn water potential) compared to the integrated and the organic treatments. The rootstock, the season, the date, and the block also had a significant influence on the pre-dawn water potential. Boerner showed a significantly higher level of water stress compared to SO4. When individual seasons were compared, 2012 showed a significantly higher level of water stress compared to 2011. An interaction between treatment and year was detected for the pre-dawn water potential. The integrated treatment showed the lowest level of water stress in 2011 and the organic treatment showed the lowest level of water stress in 2012.

Yield

Yield differed significantly among treatments and among years. The integrated treatment showed a significantly higher yield compared to the organic and the biodynamic treatments across the three growing seasons 2010 to 2012 (Table 2). Average yield of the integrated management system was 6984 kg ha^{-1} , whereas yields of the organic and the biodynamic management systems were 4276 kg ha^{-1} and 4347 kg ha^{-1} , respectively. 2010 showed the lowest average yield. Interactions between the factors treatment and year were recorded. The organic treatment showed the lowest yield in 2010 and 2012 and the biodynamic treatment showed the lowest yield in 2011 (Fig 3).

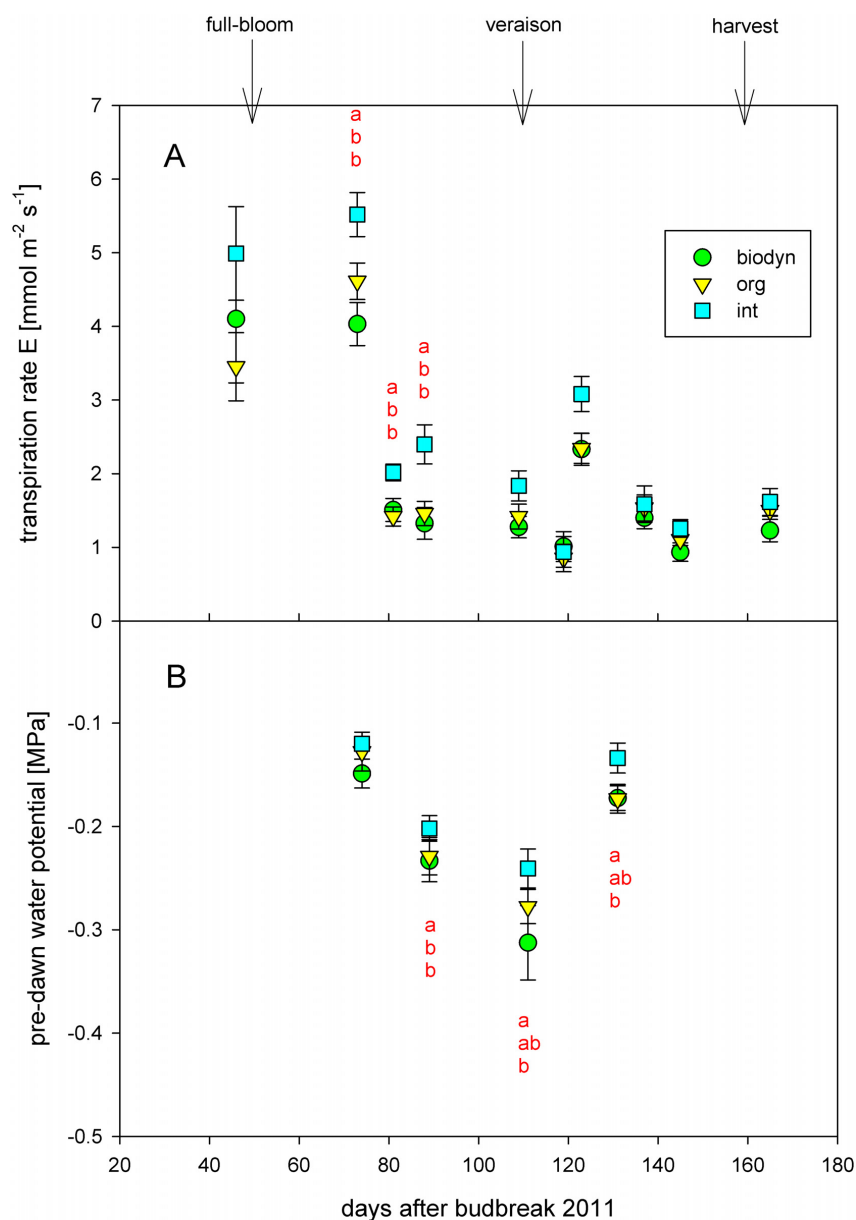


Fig 2. (A) Transpiration rate E [mmol m⁻¹ s⁻¹] and (B) pre-dawn water potential Ψ_{pd} [MPa] in 2011. Means \pm se per management system. Different letters indicate statistically significant differences (ANOVA and Tukey's test, $p < 0.05$) for the specific date. Arrows indicate full-bloom, veraison and harvest, respectively (int = integrated treatment, org = organic treatment, biodyn = biodynamic treatment).

doi:10.1371/journal.pone.0138445.g002

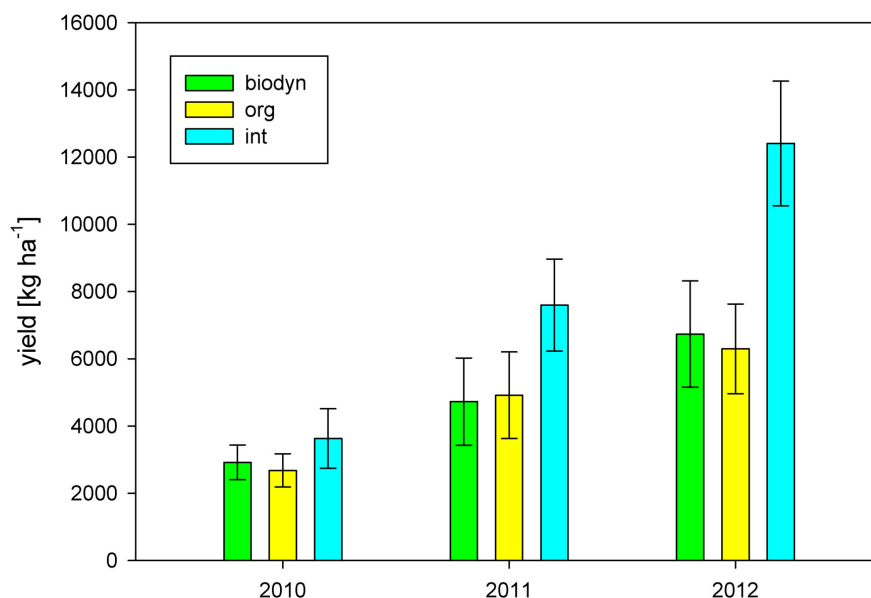


Fig 3. Yield [kg ha⁻¹] from 2010–2012. Means \pm sd per management system and year (int = integrated treatment, org = organic treatment, biodyn = biodynamic treatment).

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Leaf area to fruit weight ratio is a major indicator for vine balance of vegetative and reproductive performance. In 2012, it did not differ significantly among treatments (Table 3). The integrated treatment showed a leaf area to fruit weight ratio of 25.11 cm² g⁻¹ on average. Both, the organic and the biodynamic treatment showed a slightly increased average leaf area to fruit weight ratio of 32.41 cm² g⁻¹ and 32.94 cm² g⁻¹, respectively.

Table 3. Average values of estimated yield reduction of the organic and the biodynamic treatment compared to the integrated treatment.

| | | 2010 | 2011 | 2012 |
|--------|--|------|------|------|
| org | gravimetrically measured yield reduction [%] at harvest | 26.2 | 35.3 | 46.2 |
| | estimated yield reduction caused by downy mildew [%] | 11.2 | 0 | 6 |
| | estimated yield reduction caused by berry weight [%] | 5.9 | 8.5 | 1.1 |
| | estimated yield reduction caused by bunch weight [%] at veraison | - | - | 16.6 |
| biodyn | gravimetrically measured yield reduction [%] at harvest | 19.6 | 37.8 | 44.5 |
| | estimated yield reduction caused by downy mildew [%] | 10.3 | 0 | 3.2 |
| | estimated yield reduction caused by berry weight [%] | 2.7 | 8.5 | 1 |
| | estimated yield reduction caused by bunch weight [%] at veraison | - | - | 24.8 |

Yield reduction [%] is calculated from gravimetrically measured yield at harvest, yield reduction by downy mildew is estimated according to EPPO-guideline, yield reduction by berry weight is estimated taking into account average berry weight before harvest and yield reduction by cluster weight is estimated according to differences in cluster weight at veraison in 2012 (org = organic treatment, biodyn = biodynamic treatment).

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Average single berry weight during ripening differed significantly among treatments. The integrated treatment showed a significantly higher berry weight. Average single berry weight was also influenced by the sampling date during ripening and the rootstock. Boerner showed a significantly lower berry weight compared to SO4 (Fig 4A–4C).

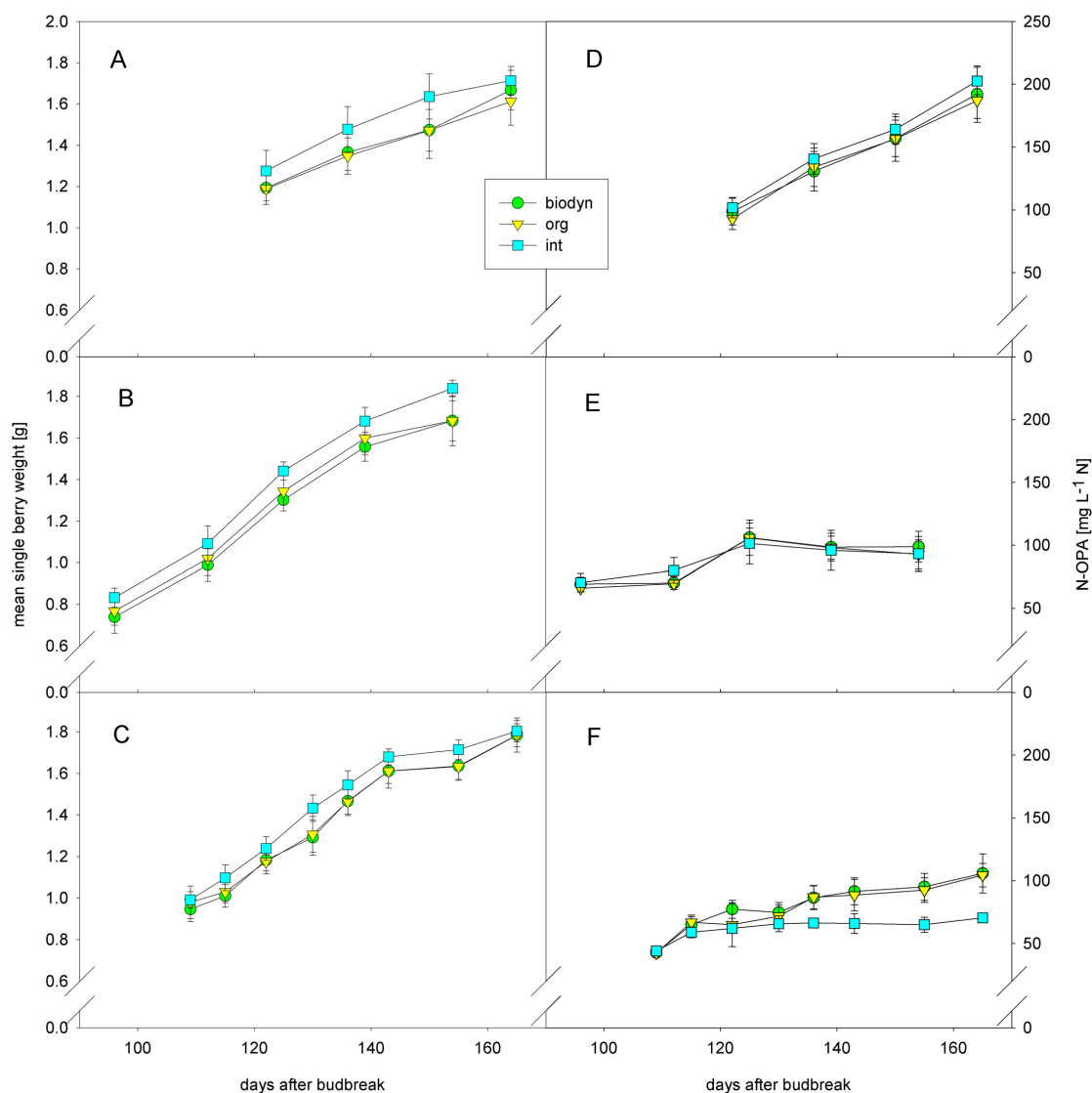


Fig 4. Mean single berry weight [g] in (A) 2010, (B) 2011, (C) 2012 and α -amino acid content (N-OPA) [mg L⁻¹ N] in (D) 2010, (E) 2011 and (F) 2012. Means \pm sd per management system and year (int = integrated treatment, org = organic treatment, biodyn = biodynamic treatment).

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Average cluster weight at veraison differed significantly among treatments in 2012. The integrated treatment showed significantly higher cluster weight (122.29 g) compared to the organic and the biodynamic management. Under organic and biodynamic management, average cluster weights were 101.94 g and 91.92 g, respectively. Interactions between treatment and rootstock occurred. The integrated treatment showed the highest average cluster weight for Boerner and the organic treatment showed the highest average cluster weight for SO4. Cluster length of representative clusters did not differ significantly among treatments in 2012. Cluster compactness was assessed as the quotient of cluster weight and cluster length. The integrated treatment showed significantly higher cluster compactness compared to organic and biodynamic management ([Table 2](#)).

Disease Incidence and Severity

The monitoring results for the infestations of downy mildew, a heterothallic oomycete, on grapes after flowering in 2010 and 2012 showed a significantly higher rate of infection in the organic and the biodynamic treatments, whereas hardly any infection of downy mildew on grapes of the integrated treatment was recorded ([Table 2](#)). The organic and the biodynamic treatments showed an average rate of infection of 2.02 and 1.89, respectively. In the integrated treatment the average rate of infection observed was of 1.02. The year had a significant influence on infection of downy mildew. In the dry season of 2011, no symptoms in any of the treatments were observed. Therefore, an interaction between treatment and year occurred. The organic treatment had the highest disease incidence of downy mildew in 2010 and 2012, respectively.

Disease incidence of Botrytis in this study differed significantly between the integrated and the biodynamic treatments ([Table 2](#)). The biodynamic treatment showed a significantly higher infection rate with an average value of 4.82, whereas the integrated treatment showed an average infection rate of 4.49. The block and the year had a significant effect on the infestation with Botrytis. In 2010 and 2011 disease frequency of Botrytis was high compared to 2012. Interactions between treatment and year were observed. The integrated treatment showed the lowest infection rate in 2010 and 2011 and the organic treatment showed the lowest infection rate in 2012.

The integrated treatment showed a significantly higher disease frequency of sour rot compared to the other treatments. The year had a significant influence on the infection of sour rot. There was an interaction between treatment and year, as no sour rot was detected in any treatment in 2012 ([Table 2](#)).

Winegrape Quality

α -amino-acid content (N-OPA) differed significantly among treatments during ripening. The biodynamic treatment showed significantly higher values compared to the integrated treatment. N-OPA also differed significantly among years, blocks, rootstocks and dates of the maturity sampling during ripening. There was a clear interaction between treatment and year ([Fig 4D–4F](#)). In 2011 and 2012 the biodynamic treatment showed the highest amount of α -amino-acids in berries during ripening and at harvest, whereas the integrated treatment showed the highest α -amino-acid content in 2010 where values were generally higher.

pH, total acidity [g L^{-1}] and total soluble solids [$^{\circ}\text{Brix}$] did not differ significantly among treatments ([Table 2](#)). All three parameters differed among dates during ripening and pH and total acidity differed among years ([S2 Fig](#)).

Discussion

Growth

Growth and vigor expressed as lateral leaf area, LAI during ripening, pruning weight, and relative chlorophyll content in leaves was evidently reduced under organic and biodynamic management. Leaf area of the organic and the biodynamic treatments was sufficient to provide an adequate assimilation surface, because for a vertical shoot positioning system as it was applied here LAI values of 1.5 up to 3 are within the desired range [90]. Pruning weight of all treatments ensured a sufficient growth [91]. Hofmann, Corvers, Kauer and Meißner [52–55] report a reduction in pruning weight of the organic plots in different trials comparing conventional and organic viticulture under the same climatic conditions. Granstedt and Kjellenberg [32] observe a reduced number of side stems of potato plants applying biodynamic compared to conventional agricultural practices. This is in accordance with the reduced lateral leaf area of the organic and biodynamic treatments in this study.

Nitrogen levels of all treatments at full-bloom and veraison were within the desired range [92,93]. The organic and the biodynamic treatments showed both higher nitrogen content in the soil [Nmin] and higher nitrogen content in the leaf tissue [%] at veraison. Therefore nitrogen content in the soil and in the leaf tissue cannot account for the reduction in growth and vigor of the organic and the biodynamic treatments. This is unexpected and might be due to the effect of legumes in the cover crop (Wolff-mixture) of the organic and the biodynamic treatments. Because the soil was generally tilled shortly before flowering, it might also explain why no differences in nitrogen content at full-bloom were observed among treatments. Even though the integrated treatment received the addition of mineral fertilizer, it showed significantly lower nitrogen content in the soil and lower nitrogen content in the leaf tissue at veraison in comparison to the other two treatments. Interactions between treatment and year reveal that the organic treatment showed higher nitrogen content in soil and leaf tissue at veraison in 2010 and 2011, where it also showed a higher pruning weight in comparison to the biodynamic treatment, whereas in 2012 the biodynamic plots showed higher nitrogen content in soil and leaf tissue at veraison as well as higher pruning weight in comparison to the organic treatment. In the case of the integrated treatment the nitrogen content in the soil and in the leaf tissue seems to be decoupled from vigor and pruning weight.

Observed magnesium levels are in the required range of 0.21 to 0.34% in the leaf tissue during the growing season [92]. Magnesium content in the integrated treatment is slightly higher compared to the organic and biodynamic systems at veraison. Bitter salts were applied in the integrated treatment at 08/13/10, 07/11/11 and 07/26/11 and magnesium nitrate fertilizer (Magnisal™) was applied at 08/02/12 and 08/14/12 (S4 Table). This might be an important parameter since magnesium is needed for chlorophyll composition. Since addition of magnesium in the integrated treatment occurred around veraison, this might be one reason why chlorophyll content did not differ among treatments at full-bloom. The integrated treatment showed both significantly higher magnesium content at veraison and significantly higher chlorophyll content at veraison and harvest.

Phosphorous and potassium contents in grapevine leaves under different management systems did not show any relevant differences in this study (data not shown).

Assimilation rate, transpiration rate and stomatal conductance are significantly higher in the integrated treatment in the three growing seasons 2010–2012. The changes in physiological performance of the organic and biodynamic plots especially under dry conditions after full-bloom in 2010 and 2011 (Fig 2) might account for the observed changes in growth and vigor. It can be deduced that the integrated treatment had higher whole-plant assimilation and transpiration, because it showed higher lateral leaf area and higher LAI as well as higher assimilation

rate, transpiration rate and stomatal conductance. Interactions between treatment and year for the assimilation rate are similar to the interactions that occurred for the indirect chlorophyll content at veraison. The organic plot showed the lowest assimilation rates and the lowest indirect chlorophyll content at veraison in 2010, whereas the biodynamic treatment showed the lowest assimilation rates and the lowest indirect chlorophyll content at veraison in 2011 and 2012, respectively. These two parameters seem to be clearly linked.

One hypothesis is that the different types of cover crops used in this study influence water availability in the soil and thus physiological performance, growth and vigor and cause interactions with the root systems of the vines. Pre-dawn water potential, a good indicator for water stress under humid climatic conditions [94], was lower under organic and biodynamic management, although just the biodynamic treatment differed significantly and interactions between treatment and year occurred. Monteiro and Lopes [95] report a decrease of pruning weight due to cover cropping in the third year of a trial comparing cultivated soil to the application of cover crops. Lopes et al. [96] discovered the transpiration rates per unit leaf area of some cover crop species to be about three times as high as those measured on grapevine leaves. The vigor and growth of the grapevines may not only be influenced by the water uptake of the Wolff-mixture in comparison to the grass mixture, but nutrient competition between cover crop and vines may also influence its chlorophyll content and growth. Due to the different cover crops there might also be a different distribution of soil moisture and therefore root development of the vines might be influenced [95,97]. Other interactions between plants of the cover crop and vines may be held responsible for changes in growth and physiological performance. Another important factor that might influence growth and vigor of the different treatments in this study are plant growth regulators such as gibberellic acid, cytokinin and especially auxin that is involved in the lateral inhibition process. Maybe differences in the root system or the water availability in the soil might account for different levels of these plant growth regulators in plant tissues under differing management systems. Investigation of available soil water in the different treatments on one hand and xylem sap flow on the other hand might provide a better insight of the relation between water potential and physiological performance of the treatments. Differences in xylem or leaf anatomy under the different treatments as a reaction to different water availability or different root distribution in the soil might as well account for differences in growth and physiological activity.

Furthermore, copper used as active ingredient in spraying agents against downy mildew might possibly influence physiological performance of organically and biodynamically grown vines and thus growth [98,99]. In this study excessive copper exposure in soils cannot account for the changes observed among the different management systems, since copper content in the soil did not differ significantly when determined in 2012 [data not shown]. Amounts were of 73.8 to 75.5 mg kg⁻¹ of soil and thus well below the copper contents in soils considered harmful for grapevines. Some studies confirm metabolic and physiological changes of *Vitis vinifera* leaves exposed to Bordeaux mixture containing copper sulphate [100,101]. However, amounts of copper applied against downy mildew in this study were low (maximum 500 g per spraying event; S5 Table). The possible impact of spraying agents containing copper used in this study (Funguran, Funguran Progress and Cuprozin; S5 Table) on physiological performance of leaves should be further investigated to determine to which extent it impacts vine growth in comparison to systemic fungicides.

Yield

Yield was significantly reduced under organic and biodynamic management in this trial. When yield was compared to the average yield of the growing area [102], the yield of the experimental

trial followed similar patterns. According to previous studies yield under organic management seems to decrease except for legumes and perennial fruits such as apple, pear and peach. For most of the studies conducted in viticulture a yield decrease under organic management was observed [52–57]. The system in this study is also a legume-based system in which nitrogen supply of the organic and biodynamic treatments is ensured by a cover crop rich in legumes. The nitrogen supply of the vines cannot account for the observed yield differences because the biological systems showed higher nitrogen contents in the soil during the three growing seasons 2010–2012 and higher nitrogen content in leaves at veraison. No yield differences between the organic and the biodynamic system in our study were observed. Danner [56] reports a decrease of yield under biodynamic compared to organic management. This could not be confirmed here.

In 2012, cluster weight and cluster compactness were significantly reduced under organic and biodynamic management. Differences in nutrient supply, physiological performance, vigor as well as water availability may have caused these differences. Reproductive development of *Vitis vinifera* is highly sensitive to vine water status [103]. Water deficits early in the season were shown to result in decreases in yield and cluster weight. If early season water deficit occurred over two or more years, the number of grape clusters per vine and the cluster weight were reduced and both factors contributed to yield decreases [103]. In this study, two of the three consecutive seasons showed a decreased transpiration rate in the organic and the biodynamic treatments, especially between bloom and veraison (Fig 2). This decreased transpiration rate might have contributed to the reduction of cluster weights in the respective treatments. The period from initiation to maturation of the grape encompasses two growing seasons [104,105]. This is why water deficits may simultaneously affect more than one reproductive process and influence not only cluster weight, berry weight and yield of one year, but also primordia that highly determine yield of the subsequent growing season. It might be one reason for lower cluster weight and lower cluster compactness in the organic and the biodynamic treatments and might simultaneously have influenced yield of the respective subsequent year. Cluster number might be another very important parameter to better understand the reasons and mechanisms of the yield differences in the different management systems.

Berry weight differed significantly among treatments. The integrated treatment showed significantly higher average single berry weight during ripening and at harvest compared to the organic and the biodynamic treatments. This is in accordance with Linder [58] (*Vitis vinifera* L. cv. Chasselas), Pool and Robinson [57] (*Vitis labrusca* cv. Elvira), and Meißner [55] (*Vitis vinifera* L. cv. Riesling), who also detected a reduction in berry weight under organic viticulture. Concerning other crops, organically grown tomatoes also showed smaller mass [25]. Differences in berry weight among treatments were most evident in the dry year 2011. This might be due to lower leaf gas exchange of the organic and the biodynamic treatments after full-bloom [106]. Since there is evidence that water deficit during the period after flowering severely reduces berry weight in grapevines [107,108], it might account for the reduced berry weights observed in the organic and the biodynamic treatments, respectively.

In 2010 and 2012, it was observed that the plots under organic and biodynamic management displayed a higher disease incidence of downy mildew with an increased severity. This could primarily be due to the use of copper and plant strengtheners in the organic and the biodynamic plots (S5 Table) as opposed to the systemic fungicides that were applied in the integrated treatment (S4 Table). Danner [56] did not observe differences in disease frequency of downy mildew among integrated, organic and biodynamic viticulture in Austria (*Vitis vinifera* L. cv. Grüner Veltliner) from 1979–1983. In that study wettable sulfur, extracts of horsetail (*Equisetum arvense*), valerian (*Valeriana officinalis*) and stinging nettle (*Urtica dioica*), alkali silicates (water glass) and calcium oxide (extracted from algae) were used as plant protection

agents and plant strengtheners in the organic and the biodynamic treatments, whereas in this study wettable sulfur, water glass, Vitisan, copper and Mycosin VIN were applied ([S5 Table](#)).

Interactions between treatment and year for the parameters yield and disease incidence of downy mildew show similar patterns. In the two growing seasons 2010 and 2012, where downy mildew occurred, the organic management system showed higher disease incidence and lower yields than the biodynamic treatment. In 2011, where downy mildew was not detected in any of the systems, the biodynamic treatment showed the lowest yields.

Compared to the integrated treatment average yield reduction is 35.9% in the organic and 34% in the biodynamic treatment ([Table 3](#)). These yield reductions can be partially explained by the reduced cluster weight, the reduced berry weight and the increased disease frequency of downy mildew. Disease frequency of downy mildew and reduced cluster weight can account for 28% out of 44.5% of yield loss in the biodynamic treatment and can account for 22.6% out of 46.2% of the yield loss in the organic treatment in 2012.

Disease frequency of downy mildew, single berry weight and cluster weight cannot account for the entire yield reduction in the organic and the biodynamic treatments which occurred from 2010–2012 ([Table 3](#)). One weakness of this assessment may be that disease frequency of downy mildew was estimated at bunch closure and not shortly before harvest. The former assessment time was chosen because the shriveling of infested bunches make detection of the disease more difficult later in the season. In comparing the season 2010 to 2012, it was observed that in 2012 a higher yield loss in the biological systems occurred. In 2010 the infestation of downy mildew took place much earlier in the growing season. We can therefore deduce that more compensation occurred and that we detected a similar rate of infection as in 2012, but observed less yield reduction. The reduced cluster weight of the organic and the biodynamic treatments measured at veraison in 2012 can be partially held responsible for the yield reduction of the respective systems. We do not know if the number of bunches per shoot were similar among the management systems and we cannot quantify the yield loss due to Botrytis shortly before harvest. These two factors may highly determine yield of the management systems. Number of clusters per shoot as well as average cluster weight, average number of berries per cluster, average berry weight and average number of shoots per vine should be determined in the future to provide a more precise idea of the reproductive growth cycle in the different treatments.

Winegrape Quality

Winegrape quality encompasses not only berry chemical traits, but also health status of the grapes and nutrient contents for ensuring successful yeast nutrition [[109](#)]. No differences in berry quality parameters such as total soluble solids, total acidity and pH during ripening and at harvest occurred among treatments. Many other studies confirm that organic and biodynamic viticulture, respectively, have little influence on grape composition [[10,52,54,56,58,61](#)]. Organically grown tomatoes [[25,26](#)] or other organically grown fruits such as strawberries [[18](#)] or apples [[17](#)], in contrast, showed higher quality. This might be highly dependent on the culture, management and physiological response of the plant. Leaf-area-to-fruit-weight-ratio in 2012 did not differ significantly among treatments in this study. Leaf-area-to-fruit-weight-ratios calculated in this study are high in comparison to values from other cultivars under semi-arid conditions [[85,110](#)], but varieties such as Gewürztraminer under cool climate conditions showed a high leaf-area-to-fruit-weight-ratio, too [[111](#)]. The fact that no differences in leaf-area-to-fruit-weight-ratios among treatments were observed might be one reason why treatments did not differ significantly in major berry quality traits. Another reason for this might be that physiological performance after veraison which influences the maturity of the

fruit [107] did not differ highly among treatments (Fig 2). Nonetheless other berry quality parameters such as phenol content or aroma components might differ among the viticultural management systems because of differences in vigor. It should be further investigated as to whether grapes of different management systems differ in berry quality parameters highly linked to light interception by the canopy and translucency of the bunch zone [112].

Disease frequency of Botrytis was significantly increased in the biodynamic treatment compared to the integrated treatment where botryticides were applied. The differences in the management between the integrated and the biodynamic treatment include soil management, cover crop, plant protection strategy and the application of the biodynamic preparations. This means that the application of the preparations cannot entirely account for the observed differences, since the organic and the biodynamic treatments did not differ significantly in disease frequency of Botrytis in this trial. Moreover, the differences in plant protection strategy, e.g. the application of botryticides in the integrated management system, cannot entirely account for the observed differences since the integrated and the organic plot do not differ significantly either. Danner [56], in contrast, reports a higher disease frequency of Botrytis for organic management compared to conventional and biodynamic management from 1979–1983 in Austria (*Vitis vinifera* L. cv. Grüner Veltliner).

Once Botrytis attacks the berries there is the risk of further fungi or bacteria entering the cracked barrier of the berry skin. One of the most frequent pathogens that severely endanger fruit and wine quality are acetic acid bacteria which cause sour rot [112,113]. Disease frequency of sour rot was significantly increased in the integrated treatment in 2010 and 2011, where sour rot on bunches occurred. One reason for this might be that copper, which was applied as a plant protection agent in the organic and the biodynamic plots until veraison (S5 Table), has a negative impact on growth of acetic acid bacteria which cause sour rot. In 2011 the monitoring results were confirmed by the gravimetric determination of the amount of berries per vine affected by sour rot. The integrated treatment showed a significantly higher amount of infected yield (data not shown). Still further research is needed to verify whether copper may account for the observed differences concerning sour rot.

The biodynamic treatment showed a significantly higher content of primary amino acids in healthy berries during maturation compared to the integrated treatment. At harvest in 2010 all treatments showed sufficiently high concentrations of primary amino acids over 140 mg N L⁻¹ to support completion of fermentation [88]. In 2011 contents of primary amino acids were generally low for all treatments. The organic and the biodynamic treatments showed a higher content of primary amino acids in 2012 compared to the integrated treatment. This may be partially due to the high yield loss in the organic and the biodynamic treatments in 2012, which was highest in the seasons of interest (Table 3). One reason for the lowest concentration of N-OPA in healthy berries of the integrated treatment might be the application of systemic fungicides. Oliva et al. [114] showed that the application of certain systemic fungicides significantly reduces total amino acid content as well as up to 11 out of 16 analyzed amino acids in grapes (*Vitis vinifera* L. cv. Monastrell). Especially fungicides that contained famoxadone or fenhexamid decreased the amino acid concentration in grapes. Teldor which contains fenhexamid as an active agent against Botrytis was applied once in 2010 and 2012, respectively, and twice in 2011 in the integrated treatment. The concentrations that were applied were slightly dependent on the phenological stages of the vines (S4 Table), but corresponded to the ones of the study by Oliva et al. [114]. A decrease in amino acid concentration in the juice might not only have implications on the success of alcoholic fermentation, but may also affect wine aroma and other beneficial effects such as protein synthesis [115]. However, the fungicide application alone cannot account for the observed differences in N-OPA, because the organic treatment did not differ significantly in N-OPA from the integrated treatment. The amount of

healthy berries might as well have influenced N-OPA since the integrated and the biodynamic treatment also differed in disease frequency of Botrytis. On one hand the application of botryticides in the integrated treatment lowered the infestation with Botrytis, but might on the other hand be partially responsible for the decline in amino acid content in berries during maturation. An interaction between the effect of the fungicide and the amount of healthy berries might have caused the observed differences in N-OPA. Another factor that is likely to have influenced the amount of Botrytis and the amount of available α -amino acids in the berries of the different treatments is the nitrogen content in the soil that was reflected in the nitrogen content in the leaf tissue. It should be further investigated if enzymes that share in conversion of nitrogen in the plant such as nitrate reductase show different activities in vines of the different treatments.

Conclusions

Growth and yield of grapevines under organic and biodynamic management decreased in comparison to the integrated treatment in this study, whereas fruit quality was not affected by the management system. Use of biodynamic preparations had little effects on vine growth and yield.

Since physiological performance was significantly higher under integrated management, it can be deduced that it influenced both growth, cluster weight, and berry weight and therefore yield levels. Soil management and fertilization strategy are likely to regulate physiological performance of the vines. Whether the changes in physiological performance occur due to hydraulic or chemical signals, such as phytohormones, should be further investigated. The discovery of reduced physiological performance of organically and biodynamically grown grapevines under field-conditions might potentially provide hints for further research on physiological performance of other organically grown perennial crops to better understand and further develop organic management strategies. Since a reduction of physiological performance in the organic and the biodynamic treatments occurred most evidently after full-bloom, organic and biodynamic growers should minimize water consumption of the cover crop in this period through mulching or rolling, because in this period berry size is determined and limited water availability might cause a reduction in cluster weight of the current and the subsequent year.

Nitrogen levels in the soil and in leaf tissues were also affected by the management system, but since the organic and the biodynamic treatments showed higher nitrogen levels, this factor cannot account for the observed reduction in growth and yield of the respective treatments. Nitrogen supply in the organic and the biodynamic treatments has been successfully ensured through cover crop management and compost addition.

Plant health differed significantly among treatments in this study due to the different plant protection strategies of the treatments investigated. In two out of three growing seasons disease incidence and severity of downy mildew in the organic and the biodynamic treatments partially accounted for yield reduction in the respective treatments. A stringent organic plant protection strategy with narrow intervals of spraying events especially in wet periods throughout the growing season is crucial to guarantee yield and fruit quality of grapevines.

Plant protection strategy also influenced nutrient status of the vines. Magnesium content in leaf tissues at veraison was significantly higher in the integrated treatment most likely due to the application of bitter salts. To which extend the higher magnesium content in the integrated treatment at veraison influenced physiological performance is subject of further research. Nonetheless, organic and biodynamic winegrowers should ensure sufficient magnesium supply to potentially enhance chlorophyll content and physiological performance of grapevines.

Since a growth reduction under organic and biodynamic management was observed in this study, further research on the microclimate in the bunch zone and secondary metabolites in

berries related to radiation interception and translucency of the bunch zone should be conducted. Furthermore, sensory characteristics of the wines from the differing management systems should be compared.

Supporting Information

S1 Dataset. The underlying dataset of the trial.

(ZIP)

S1 Fig. Data of weather conditions during the seasons (A) 2010, (B) 2011, and (C) 2012.

Daily average temperature [$^{\circ}\text{C}$] and daily rainfall [mm]. Arrows indicate budbreak, full-bloom, veraison and harvest, respectively.

(TIF)

S2 Fig. Maturity sampling during the seasons (A) 2010, (B) 2011, and (C) 2012. Total soluble solids [$^{\circ}\text{Brix}$], total acidity [g L^{-1}], and pH. Means \pm sd.

(TIF)

S1 Table. Results of the balanced fixed factorial analysis of variance (ANOVA with factors treatment and block) for the analysis of the soil samples in 2010 before data collection started [116]. *, ** and *** indicate statistical significance ($p < 0.05$, $p < 0.01$ and $p < 0.001$) of the main effects determined by ANOVA (ns = not significant). Means \pm sd per management system (int = integrated treatment, org = organic treatment, biodyn = biodynamic treatment).

(DOC)

S2 Table. Analysis of residues of systemic plant protection agents on bunches in 2009.

int = integrated treatment, org = organic treatment

(DOC)

S3 Table. Components of the Wolff-mixture used as cover crop in the organic and the biodynamic treatment.

(DOC)

S4 Table. Pest and disease management of the integrated treatment.

(DOC)

S5 Table. Pest and disease management of the organic and the biodynamic treatment.

(DOC)

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Author Contributions

Conceived and designed the experiments: RK MS. Performed the experiments: JD. Analyzed the data: JD. Contributed reagents/materials/analysis tools: JD RK MS ST MF. Wrote the paper: JD MF ST MS RK. Helped choosing methods used for data collection: ST. Helped with Equipment for data collection: ST.

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Chapter 5 Organic and Biodynamic Viticulture affect Soil, Biodiversity, Vine and Wine Properties: a Systematic Quantitative Review⁴

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Organic and Biodynamic Viticulture Affect Biodiversity and Properties of Vine and Wine: A Systematic Quantitative Review

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Abstract: Demand for organically grown crops has increased exponentially in the last few decades. Particularly in the wine sector, organic and biodynamic management systems are gaining more and more importance, with some of the most prestigious wineries converting to organic or biodynamic viticulture. The purpose of this study was to review evidence comparing effects of conventional, organic, and biodynamic viticulture on soil properties, biodiversity, vine growth and yield, disease incidence, grape composition, sensory characteristics, and wine quality. Only studies with representative field replicates or studies with a representative number of samples were included. Soil nutrient cycling was enhanced under organic viticulture, especially after conversion was completed. Cover crop mixtures used, compost application, and the absence of herbicides might be factors that account for higher biological activity in organically and biodynamically managed soils. Seventeen out of 24 studies observed a clear increase in biodiversity under organic viticulture on different trophic levels. Plant protection regime and cover crop mixtures mainly determine higher biodiversity in organic and biodynamic viticulture. Organic and biodynamic treatments showed 21% lower growth and 18% lower yield compared to conventional viticulture. The decrease of growth and yield under organic and biodynamic viticulture was not correlated to the growth or yield level under conventional viticulture. A decrease in soil moisture content and physiological performance (assimilation rate, transpiration rate, and stomatal conductance) under organic and biodynamic viticulture is likely to be responsible for the lower growth and yield in the respective management systems. Juice total soluble solids concentration did not differ among the different management systems. No overall differences in berry composition or juice and wine quality among management systems could be observed. By describing different hypotheses concerning the effects of organic and biodynamic viticulture, this review and meta-analysis provides helpful guidance for defining further research in organic agriculture on perennial, but also on annual, crops.

Key words: biodiversity, cover crop, crop level, floor management, grape composition, vegetative growth, wine composition

The production of organically grown crops has increased exponentially in the last few decades based on consumer demands for healthy food as well as environmentally friendly farming practices (Yiridoe et al. 2005). Current agricultural and environmental policies are reacting to these demands with initiatives limiting the use of synthetic pesticides, thus promoting organic farming (Vidal and Kelly 2013, Wysling 2015,

Kucera 2017). The controversial debate on the ban of glyphosate, the main ingredient of Monsanto's best-selling herbicide "Roundup," in the European Union (EU) has lately made organic farming the center of attention again (Neslen 2017).

The start of organic agriculture that developed almost independently in the German- and English-speaking world dates back to the beginning of the last century. The first movements toward organic farming were developed from a reaction to ecological and soil-related issues, but also to economic and social problems that occurred during the two world wars. Acidification of soils, loss of soil structure, soil fatigue, decrease of seed and food quality, and an increase of plant and animal diseases were attributed to the chemical-technical intensification of agriculture (Vogt 2000). In addition, yield levels in Germany decreased drastically in the 1920s in comparison to the years before World War I, even though the use of mineral fertilizers increased. The early movements toward organic agriculture focused on improved soil fertility while reducing the use of mineral fertilizers, and aimed to create a more sustainable form of agriculture while still producing high quality crops. The different forms of organic agriculture have evolved with time and now incorporate knowledge about biologically stabilized soil structure, rhizosphere dynamics, and systems ecology (Vogt 2000, 2007). Research on the respective management systems started mainly after World War II with the establishment of some long-term field trials

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comparing different agricultural management systems (Stinner 2007). In viticulture, organic and biodynamic management approaches were initially applied in the late 1960s, with research on organic viticulture starting soon after (Danner 1985).

Land used for organic agriculture increased from 11 million ha in 1999 to 43.7 million ha in 2014, which is ~1% of global agricultural land. At the same time, the organic market size increased from US\$15.2 billion in 1999 to \$80 billion in 2014 (Lernoud and Willer 2016). Compared to total agriculture, perennial cropland has a much higher share in organic management (Lernoud and Willer 2016). In viticulture, 316,000 ha of grapes are grown organically, which is a 4.5% share of the global grapegrowing area. Most of this organic grapegrowing area is located in Europe (266,000 ha). The three countries with the largest organic grapegrowing area are Spain, Italy, and France (Lernoud and Willer 2016). Worldwide, 11,200 ha of vineyards for wine production are managed according to biodynamic principles or are in conversion to biodynamic viticulture (Castellini et al. 2017). The biggest international biodynamic association is Demeter. Particularly in the wine sector, organic and biodynamic management practices are gaining more importance, with some of the most prestigious wineries converting to organic or biodynamic viticulture (Reeve et al. 2005).

The United Nations' Food and Agriculture Organization (FAO) defines organic farming as follows: "Organic agriculture is a holistic production management system which promotes and enhances agroecosystem health, including biodiversity, biological cycles, and soil biological activity. It emphasizes the use of management practices in preference to the use of off-farm inputs.... This is accomplished by using, where possible, agronomic, biological, and mechanical methods, as opposed to using synthetic materials, to fulfill any specific function within the system" (FAO 1999).

In the EU, several regulations exist to control organic farming (Regulation (EC) No. 834/2007, Regulation (EC) No. 889/2008). In Appendix II of Regulation (EC) No. 889/2008, there is a list of substances allowed in organic farming, and thus, organic viticulture. Any substance not on the list is forbidden. Furthermore, there is a regulation in the EU (Regulation (EU) No. 203/12) outlining detailed rules on organic winemaking. There are also specific national rules, for example, the restriction of copper (Cu) use in German viticulture to 3 kg/ha/yr (BVL, accessed 15 April 2019; https://www.bvl.bund.de/DE/04_Pflanzenschutzmittel/01_Aufgaben/02_Zulassung-PSM/01_ZugelPSM/psm_ZugelPSM_node.html), and several organic or biodynamic associations impose stricter rules than the EU standards (e.g., ECOVIN association in Germany imposes a maximum nitrogen (N) input of 150 kg/ha/3 yrs and allows growers to plough the soil within rows without sowing cover crop for a maximum three months during summer).

In the United States (US), the organic regulations by the US Department of Agriculture (USDA) control organic farming (USDA Organic Regulations; <https://www.ams.usda.gov/rules-regulations/organic>). The National Organic Program (NOP) develops rules and regulations for production, handling, labeling, and enforcement of organic products (USDA Organic Regulations). In contrast to EU standards, there is a National

List of Allowed and Prohibited Substances in the US (USDA National List; <https://www.ams.usda.gov/rules-regulations/organic/national-list>). Beyond that, various national and regional organic farming associations exist in the US.

In Australia, the biosecurity section of the Department of Agriculture, formerly the Australian Quarantine and Inspection Service of the Department of Agriculture, Fisheries, and Forestry of the Australian Government (AQIS), is responsible for the accreditation of the national certifying bodies according to the National Standard for Organic and Bio-Dynamic Produce (OISCC; <http://www.agriculture.gov.au/SiteCollectionDocuments/aqis/exporting/food/organic/national-standard-edition-3-7.pdf>). Appendices of this national standard contain lists of permitted materials in organic and biodynamic farming, as well as the criteria to evaluate inclusion of inputs and additives in this standard (Organic Industry Standards and Certification Committee 2015). As of 2015, there were seven approved certifying organizations in Australia. Besides these national control bodies, there are several associations, such as Biodynamic Agriculture Australia and Demeter, that set stricter rules than does the national legislation.

There is a transition period of three years in the US, EU, and Australia for becoming a certified organic or biodynamic producer. The implementation of regulations concerning organic vineyard management is described by Provost and Pedneault (2016).

Biodynamic farming abides by the same regulations as organic farming. It is a holistic agricultural system based on respect for the spiritual dimension of the living and inorganic environment (Vogt 2007). It was founded in 1924 by Rudolf Steiner and was one of the first movements toward organic agriculture (Steiner 2005). It should ideally be practiced on mixed farms, including crops and livestock, to meet the requirements of the farm as an organism as identified by Steiner in his agricultural course (Steiner 2005). The application of specific biodynamic preparations is one key element of this management system (Leiber et al. 2006), and is one essential difference in comparison to organic farming. These substances are said to stimulate soil nutrient cycling and promote photosynthetic activity of the crops and compost transformation (Masson and Masson 2013). The following biodynamic preparations are usually applied in biodynamic agriculture: horn manure and horn silica (Table 1) are diluted in water in very small quantities, stirred for one hour, and then applied to soil or plants, respectively (Masson and Masson 2013). The compost preparations (Table 1) are applied to a compost and are said to facilitate the transformation process into decay products (Masson and Masson 2013). Each of the preparations is put into compost in small quantities and the valerian preparation is sprayed over the compost heap (Masson and Masson 2013).

The aim of this meta-analysis is to summarize the outcomes of scientific trials performed on organic and biodynamic viticulture worldwide, and hence to characterize the effects of the respective management systems. This review addresses the question of whether conventionally, organically, and biodynamically managed vineyards differ in regards to

soil properties, biodiversity, vine growth and yield, disease incidence, grape composition, wine quality, sensory characteristics, and production costs. Qualitative results showing the overall effects of organic and biodynamic viticulture and quantitative results displayed as regression analyses of available data sets are presented. By describing and quantifying the effects of the respective viticultural management systems, this review provides decision support for producers, consumers, and researchers. In addition, new findings concerning the reasons for the effects of organic and biodynamic management in viticulture are described. Different hypotheses for explaining the observed phenomena are presented. Therefore, this review and meta-analysis also provides helpful guidance for defining future areas of research.

Published Data Sourcing and Selection

Literature searches of peer-reviewed published literature were conducted to find studies investigating organic and/or biodynamic viticulture. The following search terms were used in different combinations in the ISI-Web of Science and PubMed databases: organic/viticulture, biodynamic/viticulture, biodynamic/agriculture/grapes (last searched 25 Aug 2017).

Only field trials that used replicates of management treatments with representative plots or studies that used a representative number of samples were included in the review in order to avoid bias in individual studies. Data from non-peer-reviewed sources such as conference proceedings, master's theses, or doctoral dissertations were also included in this study if they met the criteria mentioned above. In Germany and Austria, some long-term studies were conducted between 1980 and 2015, whose results were published as doctoral theses in German. These findings were translated and included. In Australia, a long-term trial was conducted between 2009 and 2014 whose results were partially published as honors and master's theses, and results were included here. Furthermore, unpublished data from the Australian study and from a long-term trial in Germany were provided by the authors and were included in the review and in the meta-analysis.

This led to a total number of 84 studies included in the quantitative review, of which 64 were peer-reviewed and 20 were non-peer-reviewed.

Seventeen studies that met the criteria mentioned above and whose data sets were available were included in the quantitative meta-analysis. Data were extracted manually from the different studies. If different forms of conventional viticulture

were included in the studies, low-input conventional plots were chosen for meta-analysis. If compost was applied to the different plots, as was the case in the Australian long-term trial, means per treatment and year were calculated for plots with and without compost application. These treatment means were included in the meta-analysis. For the study by Linder et al. (2006) and by Wheeler and Crisp (2011), means per treatment over eight- and five-year periods, respectively, were available and were included. Characteristics of the studies included in the meta-analysis and meta-regressions are presented in Supplemental Table 1.

Data Analysis

Linear meta-regression analyses were performed to evaluate the quality of the correlations of several parameters (growth, yield, and total soluble solids [TSS] in juice) between conventionally and organically or biodynamically managed vineyards. By meta-regression analyses, the following question was addressed: What is the magnitude of the effect of organic and biodynamic management on vine growth (expressed as pruning weight), vine yields, and TSS in juice?

To assess whether conventional and organic/biodynamic viticulture differ in vine growth, vine yield, and TSS in juice, and whether the observed effects are consistent across all environments, analysis of variance (ANOVA) and Tukey's test (post-hoc test) were performed. The factors were treatment (conventional or organic, including biodynamic) and location of the study (United States, Europe, or Australia), and interactions between treatment and location were assessed. All statistics were carried out using the statistical software R (Ihaka and Gentleman 1996). For meta-regression analysis, as well as for ANOVA and Tukey's test means per treatment, year and variety were calculated to avoid bias among studies due to differing numbers of plots or vineyards.

For all studies considered in the meta-analysis and meta-regressions, effects and variance were estimated (Supplemental Figures 1 to 3). Studies are partially heterogenic (especially concerning the pruning weight), are limited in number, and publication bias cannot be excluded.

Management Effects on Soil

The improvement of soil fertility without any synthetic N fertilizers is a key principle of organic farming. The most important source of N as well as other nutrients in organic farming is the use of compost. It supplies the soil with organic

Table 1 Main ingredients of the biodynamic preparations 500 to 507 (adapted from Reeve et al. 2005).

| Preparation | Main ingredient | Use |
|-----------------------|--|-------------|
| Horn manure (500) | Cow (<i>Bos taurus</i>) manure | Field spray |
| Horn silica (501) | Finely ground quartz silica | Field spray |
| Yarrow (502) | Yarrow blossoms (<i>Achillae millefolium</i> L.) | Compost |
| Chamomile (503) | Chamomile blossoms (<i>Matricaria recutita</i> L. or <i>Matricaria chamomilla</i> L.) | Compost |
| Stinging nettle (504) | Stinging nettle shoots and leaves (<i>Urtica dioica</i> L.) | Compost |
| Oak bark (505) | Oak bark (<i>Quercus robur</i> L.) | Compost |
| Dandelion (506) | Dandelion flowers (<i>Taraxacum officinalis</i> L.) | Compost |
| Valerian (507) | Valerian flower extract (<i>Valeriana officinalis</i> L.) | Compost |

N, which has to be converted into inorganic N compounds to be taken up by the plants (Kauer 1994, Vogt 2007). Therefore, the stimulation of soil nutrient cycling plays a major role in organic farming as well as in organic viticulture to supply the plants with inorganic nutrients. The biological activity of the soil and the feeding activity of soil organisms are reported to increase under organic and biodynamic viticulture in comparison to conventional management (Gehlen et al. 1988, Reinecke et al. 2008, Okur et al. 2009, Freitas et al. 2011) (Table 2). The contents of organic carbon (C) and total N, phosphorus (P), and sulfur (S) did not differ among treatments (Wheeler 2006, Probst et al. 2008, Collins et al. 2015b). In contrast, P contents in the soil were reported to rapidly decrease after conversion in a long-term field trial in southern France. After seven years of conversion, authors observed a gradual increase of available P contents under organic management (Coll et al. 2011). Biodynamic and organic vineyards show a higher cumulative soil respiration, a higher content of microbial biomass C, and a higher ratio of microbial biomass C to organic C, especially after conversion (Gehlen et al. 1988, Probst et al. 2008, Okur et al. 2009, Coll et al. 2011, Freitas et al. 2011, Collins et al. 2015b). In one study conducted in southern France, soil organic matter and potassium (K) content were increased under organic viticulture (Coll et al. 2011). Two reasons for the increased contents of P and K in the soil under organic viticulture could be the increased microbial activity and the increased microbial biomass (Coll et al. 2011). In addition, organic and biodynamic treatments show lower $q\text{CO}_2$ values (Probst et al. 2008, Freitas et al. 2011). Low $q\text{CO}_2$ values indicate high microbial substrate-use efficiency (Probst et al. 2008). This is in accordance with the results of Mäder et al. (2002), who showed that higher microbial substrate-use efficiency in combination with a higher availability of soil organic matter to soil microorganisms is a characteristic result of organic farming. Yet indicators of microbial activity in the soil are strongly dependent on the vineyard location and its management. The positive effects of organic and biodynamic vineyard management on soil microbial properties are reported to increase together with the time-span since conversion (Probst et al. 2008, Coll et al. 2011). Mineralized N content in the topsoil layer did not differ among organic, biodynamic, and integrated viticulture in a field trial in Germany in the first three years of conversion (Meißner 2015). Integrated farming is an approach that promotes sustainable farming by using all possible tools and techniques to reduce input of chemicals. Polluting inputs are minimized and resources are used sustainably (Ente Nazionale Italiano di Unificazione 2009). In the same trial where results of Meißner (2015) were obtained, mineralized N content in the topsoil layer was reported to increase under organic and biodynamic management after the first four years of conversion (Döring et al. 2015). This implies that stimulation of soil nutrient cycling by compost application, the implementation of cover crop mixtures with a wide range of species, including legumes, and denial of mineral fertilizers, as practiced in the organic and biodynamic treatments, takes some years to make an impact on

N levels and on microbial activity in the soil. This again is in accordance with other findings concerning soil microbial properties under organic management (Coll et al. 2011). As mentioned above, microbial biomass C also increased under organic and biodynamic viticulture after conversion in a long-term field trial in Australia (Collins et al. 2015a). Soil quality such as microbial efficiency and mineralized N content in the soil did not differ between organic and biodynamic treatments (Reeve et al. 2005, Döring et al. 2015, Meißner 2015) (Table 3).

Increased soil compaction under organic viticulture was reported in a long-term trial in southern France (Coll et al. 2011). This might be due to a higher frequency of plant protection applications under organic farming.

Cu products are among the oldest plant protection agents and represent an important part of the plant protection strategy against downy mildew (caused by *Plasmopara viticola*) in organic viticulture. However, Cu is accumulated in the soil, and high Cu content in vineyard soils is mainly due to anthropogenic inputs in past decades (Probst et al. 2008, Strumpf et al. 2009). Cu inputs from 1890 to 1940 were typically up to 50 kg/ha/yr in viticulture (Strumpf et al. 2011). In long established winegrowing regions with long-term Cu application, Cu levels in the soil are higher compared to areas where viticulture was developed within the last three to five decades (Strumpf et al. 2009). There is no direct correlation between Cu content in the soil and its plant availability (Steindl et al. 2011). Cu content in grapes is low even if Cu content in the soil is high (Strumpf et al. 2009). Cu levels in viticultural soils have impact on total C, enzyme activities, and biodiversity, especially on earthworm abundance in the soil (Paoletti et al. 1998, Mackie et al. 2013). Although amounts of Cu used for plant protection in organic viticulture are higher compared to the amounts usually used in conventional viticulture, organically managed vineyard soils in France, Croatia, and Germany did not have a higher Cu content compared to their conventional counterparts (Probst et al. 2008, Coll et al. 2011, Strumpf et al. 2011, Radić et al. 2014). Beni and Rossi (2009), in contrast, observed higher total Cu contents under organic farming after nine years in Italy. In an Italian study on organic viticulture, Cu amounts in soils, on berries, and in wines were below the maximum residue levels (Provenzano et al. 2010). Since vineyard soils under conventional, organic, or biodynamic management did not show differences in Cu levels in most of the studies, there were no negative implications for earthworms in the soil (Strumpf et al. 2011).

Management Effects on Biodiversity

Biodiversity in agroecosystems provides multiple ecological services beyond food production that lead to increasing internal regulation of food production (Altieri 1999). Results concerning biodiversity in annual crops and grasslands support the hypothesis that organic farming enhances biodiversity. There is evidence that organic agricultural methods increase species richness and abundance compared to conventional farming systems (Bengtsson et al. 2005, Hole et al. 2005). On average, species richness was 30% higher,

Table 2 Effects of organic viticulture in comparison to conventional viticulture grouped by different fields of interest.

| Field of interest/ parameters | Effect compared to integrated/ conventional management | Management system | References |
|---|---|----------------------|--|
| Soil | | | |
| Biological activity, feeding activity of soil organisms, soil organic matter, total K | Increase | Org | Coll et al. 2011, Freitas et al. 2011, Gehen 1988, Okur et al. 2009, Reinecke et al. 2008 |
| Soil organic C content, total N, P, S, microbial biomass C during conversion | No difference | Biodyn | Collins et al. 2015b, Probst et al. 2008, Wheeler 2006 |
| Microbial biomass C, C_{mic}/C_{org} , soil respiration after conversion | Increase | Org biodyn | Collins et al. 2015b, Freitas et al. 2011, Gehlen 1988, Okur et al. 2009, Probst et al. 2008 |
| Soil compaction | Increase | Org | Coll et al. 2011 |
| Metabolic quotient qCO_2 | Decrease | Org biodyn | Freitas et al. 2011, Probst et al. 2008 |
| Mineralized N during conversion | No difference | Org biodyn | Coll et al. 2011, Meißner 2015 |
| Mineralized N after conversion | Increase | Org biodyn | Döring et al. 2015 |
| Cu content in soils | No difference | Org biodyn | Coll et al. 2011, Probst et al. 2008, Radic et al. 2014, Strumpf et al. 2011 |
| Cu content in soils | Increase | Org | Beni and Rossi 2009 |
| Soil moisture | Decrease | Org biodyn | Collins et al. 2015b |
| Biodiversity | | | |
| Arbuscular mycorrhizal fungi, bacterial biodiversity in topsoil, fungal diversity on leaves, shoots, and grapes, fungal species richness on bark and grapes, yeast species abundance in must | Increase | Org biodyn | Bagheri et al. 2015, Freitas et al. 2011, Hendgen et al. 2018, Morrison-Whittle et al. 2017, Radic et al. 2014, Schmid et al. 2011 |
| Fungal species richness in soil, epiphytic microbial communities on grapes, fungal community composition in harvested juice | No difference | Org biodyn | Bagheri et al. 2015, Hendgen et al. 2018, Kecskeméti et al. 2016, Morrison-Whittle et al. 2017 |
| Yeast abundance on grapes | Decrease | Biodyn | Guzzon et al. 2016 |
| Plant species richness, perennial plant species, earthworm abundance, nematode density, biodiversity and abundance of predatory mites, species richness of butterflies, biodiversity and abundance of arthropods, ladybird abundance, detritivore abundance, colembola abundance, spider biodiversity, feeding ecology of birds | Increase | Org biodyn | Caprio et al. 2015, Caprio and Rolando 2017, Coll et al. 2011, Collins et al. 2015b, Fleury and Fleury 2016, Isaia et al. 2006, Meißner 2015, Nascimbene et al. 2012, Peverieri et al. 2009, Puig-Montserrat et al. 2017, Wheeler 2006 |
| Plant diversity and abundance, plant species composition, moth biodiversity, insect pollination, spider biodiversity and abundance, biodiversity and abundance of birds | No difference | Org | Assandri et al. 2016, Brittain et al. 2010, Bruggisser et al. 2010, Nascimbene et al. 2012, Puig-Montserrat et al. 2017 |
| Endogenic earthworm density and biomass, abundance of predatory mites, biodiversity of grasshoppers, biodiversity of ladybirds | Decrease | Org | Bruggisser et al. 2010, Coll et al. 2011, Fleury and Fleury 2016, Linder et al. 2006 |
| Growth | | | |
| Ratio yield:pruning weight | No difference | Org biodyn | Collins et al. 2015b |
| Ratio leaf area:fruit weight | No difference | Org biodyn | Döring et al. 2015 |
| Pruning weight, shoot length, canopy density | Decrease | Org biodyn | Collins et al. 2015b, Corvers 1994, Döring et al. 2015, Hofmann 1991, Kauer 1994, Malusà et al. 2004, Meißner 2015, Pike 2014 |
| Leaf area index (LAI) | No difference | Org | Corvers 1994 |
| Leaf area index (LAI) | Decrease | Org biodyn | Döring et al. 2015 |
| Macronutrient supply in leaves (veraison), chlorophyll content (full-bloom) | No difference | Org biodyn | Collins et al. 2015b, Döring et al. 2015, Linder et al. 2006, Meißner 2015 |
| Nitrogen content in leaves (veraison) | Increase | Org biodyn | Döring et al. 2015 |
| Nitrogen content in leaves, nutrient supply | Decrease | Org biodyn | Danner 1985, Malusà et al. 2004 |
| Chlorophyll content (veraison), Mg and P contents in leaves or petioles | Decrease | Org biodyn | Collins et al. 2015b, Döring et al. 2015, Meißner 2015 |
| Physiological performance (A, E, gs) | Decrease | Org biodyn | Döring et al. 2015 |
| Predawn water potential Ψ_{pd} | Decrease | Biodyn | Döring et al. 2015 |
| Stem water potential before harvest | No difference | Org biodyn | Collins and Döring unpublished |
| Yield | | | |
| | Decrease | Org biodyn | Collins et al. 2015a, Corvers 1994, Danner 1985, Döring et al. 2015, Hofmann 1991, Kauer 1994, Malusà et al. 2004, Meißner 2015, Pool and Robinson 1995, Wheeler 2006 |
| | No difference | Org | Danner 1985 |

continued on next page

Table 2 (continued) Effects of organic viticulture in comparison to conventional viticulture grouped by different fields of interest.

| Field of interest/ parameters | Effect compared to integrated/ conventional management | Management system | References |
|---|---|----------------------|---|
| Yield (continued) | | | |
| Berry weight | No difference | Org | Corvers 1994, Pool and Robinson 1995 |
| Berry weight, compactness of bunches | Decrease | Org biodyn | Collins et al. 2015b, Döring et al. 2015, Linder et al. 2006, Meißner 2015, Pool and Robinson 1995 |
| Number berries per bunch, average bunch weight | Decrease | Org biodyn | Corvers 1994, Collins et al. 2015b, Döring et al. 2015 |
| | No difference | Org | Pool and Robinson 1995 |
| Disease incidence | | | |
| Disease incidence <i>Plasmopara viticola</i> | No difference | Org biodyn | Danner 1985, Pike 2014 |
| | Increase | Org biodyn | Döring et al. 2015 |
| Disease incidence <i>Erysiphe necator</i> | Increase | Org | Linder et al. 2006 |
| Disease incidence <i>Botrytis cinerea</i> | Increase | Org biodyn | Danner 1985, Döring et al. 2015 |
| | No difference | Org biodyn | Danner 1985, Meißner 2015, Pike 2014 |
| Disease incidence sour rot, root necrosis (fungal pathogens) | Decrease | Org biodyn | Döring et al. 2015, Lotter et al. 1999, Meißner 2015 |
| Winegrape quality | | | |
| Berry composition | No difference | Org biodyn | Collins et al. 2015a, Collins et al. 2015b, Danner 1985, Döring et al. 2015, Henick-Kling 1995, Hofmann 1991, Kauer 1994, Linder et al. 2006, Malusà et al. 2004, Tassoni et al. 2013, Tassoni et al. 2014, Wheeler 2006 |
| Fe and Zn in berries | Increase | Org | Coffey 2010 |
| Volatile acidity and malic acid | Increase | Org | Beni and Rossi 2009 |
| Juice acidity, citric acid in wines | Decrease | Org biodyn | Corvers 1994, Meißner 2015, Tobolková et al. 2014 |
| Juice and wine quality | No difference | Org biodyn | Danner 1985, Dupin et al. 2000, Granato et al. 2015b, Granato et al. 2015c, Granato et al. 2016, Henick-Kling 1995, Kauer 1994, Meißner 2015 |
| Juice quality (by image forming methods) | Increase | Org biodyn | Fritz et al. 2017 |
| Yeast available nitrogen | Increase | Biodyn | Döring et al. 2015 |
| | No difference | Org biodyn | Collins et al. 2015b, Meißner 2015 |
| Anthocyanin and flavonoid content in berry skin, putrescine (biogenic amine) in wines | Increase | Org | Malusà et al. 2004, Yildirim et al. 2007 |
| Polyphenol content, antioxidant potential (grapes, juice, and wine), phenolic acids, enzyme polyphenol oxidase, <i>trans</i> -resveratrol | Increase | Org biodyn | Buchner et al. 2014, Dani et al. 2007, Granato et al. 2015a, Micelli et al. 2003, Malusà et al. 2004, Nuñez-Delgado et al. 2005, Otreba et al. 2011, Rodrigues et al. 2012, Tintunen and Lehtonen 2001, Vrček et al. 2011, Yildirim et al. 2004 |
| Alcohol content, total anthocyanins, polyphenol profile grapes and wines, carotenoids, color density in wine, <i>trans</i> -resveratrol, <i>p</i> -coumaric acid, antioxidant activity, biogenic amines | No difference | Org biodyn | Bunea et al. 2012, Collins et al. 2015a, Collins et al. 2015b, Garaguso and Nardini 2015, Lante et al. 2004, Mulero et al. 2009, Mulero et al. 2010, Tassoni et al. 2013 |
| Polyphenol content, antioxidant activity in wine, Cu and Fe in wines, ascorbic acid equivalents, ferric-reducing power | Decrease | Org | Beni and Rossi 2009, Tobolková et al. 2014, Yildirim et al. 2004 |
| Sensory characteristics | | | |
| Berry sensory analysis – pulp juiciness | Increase | Org | Coffey 2010 |
| Wine sensory characteristics | No difference | Org biodyn | Danner 1985, Dupin et al. 2000, Kauer 1994, Meißner 2015 |
| Sensory attributes “floral, fruity, vegetal, complex, skunky, astringent” | Decrease | Org biodyn | Beni and Rossi 2009, Dupin et al. 2000, Henick-Kling 1995, Meißner 2015 |
| Sensory attributes “balance, full-bodied, minerality, length” | Increase | Biodyn | Beni and Rossi 2009, Meißner 2015 |
| Sensory attributes “rich, textual, complex, vibrant, spicy” | Increase | Org Biodyn | Collins et al. 2015 |
| Sensorial preference of tasting panel (ranking) | Increase | Biodyn | Beni and Rossi 2009, Henick-Kling 1995, Meißner 2015 |
| Costs and efficiency | | | |
| Production costs and operational costs, productive efficiency | Increase | Org biodyn | Danner 1985, Delmas et al. 2008, Guesmi et al. 2012, Linder et al. 2006, Santiago 2010, Santiago and Johnston 2011, Wheeler 2006, White 1995 |
| Environmental impact, total energy inputs, greenhouse gas emissions | Decrease | Org biodyn | Kavargiris et al. 2009, Villanueva-Rey et al. 2014 |

and organisms were 50% more abundant in organic farming systems, compared to conventional management in annual crops (Bengtsson et al. 2005). Still, effects differed between organism groups and landscapes, and benefits for biodiversity have not always been found. Birds, predatory insects, soil organisms, and plants showed enhanced biodiversity under organic farming, while nonpredatory insects and pests did not (Bengtsson et al. 2005, Hole et al. 2005). It is controversially discussed whether an organic whole-farm approach provides more benefits for biodiversity than the establishment of small habitats within intensively used agricultural land (Hole et

al. 2005). Further research is needed to assess the long-term effects of organic agriculture on biodiversity. Perennial cropping systems such as vineyards could be a good model for long-term studies on biodiversity since their lifespan usually comprises at least a few decades, and they often provide habitats for rare and endangered species because of their climatic peculiarities (Bruggisser et al. 2010). Thus, biodiversity in perennial systems such as vineyards can generally be very high (Isaia et al. 2006, Peverieri et al. 2009). Nevertheless, the effects of organic management on biodiversity in perennial crops have not been reviewed.

Table 3 Effects of biodynamic viticulture in comparison to organic viticulture.

| Field of interest/ parameters | Effect compared to organic management | Management system | References |
|---|---|----------------------|---|
| Soil and biodiversity | | | |
| Soil quality, mineralized N, microbial efficiency in soil, epiphytic microbial communities, arthropods | No difference | Biodyn | Döring et al. 2015, Kecskeméti et al. 2016, Reeve et al. 2005, Meißner 2015 |
| Earthworm abundance | Increase | Biodyn | Meißner 2015 |
| Growth | | | |
| Ratio yield:pruning weight | Decrease | Biodyn | Reeve et al. 2005 |
| | No difference | Biodyn | Collins et al. 2015b |
| Pruning weight, LAI ^a , leaf-area-to-fruit-weight-ratio, macronutrients in leaves, chlorophyll content, physiological performance | No difference | Biodyn | Döring et al. 2015, Meißner et al. 2015, Reeve et al. 2005 |
| Stomatal conductance | No difference | Biodyn | Döring et al. 2015 |
| | Decrease | Biodyn | Botelho et al. 2015 |
| Leaf enzymatic activity, intrinsic WUE ^a | Increase | Biodyn | Botelho et al. 2015 |
| Predawn water potential Ψ_{pd} | Decrease | Biodyn | Botelho et al. 2015, Döring et al. 2015 |
| Yield | | | |
| | No difference | Biodyn | Botelho et al. 2015, Döring et al. 2015, Meißner 2015, Reeve et al. 2005 |
| | Decrease | Biodyn | Danner 1985 |
| Clusters per vine, cluster weight, cluster compactness, berry weight | No difference | Biodyn | Döring et al. 2015, Meißner 2015, Reeve et al. 2005 |
| Disease incidence | | | |
| Disease frequency <i>Plasmopara viticola</i> , <i>Botrytis cinerea</i> | No difference | Biodyn | Danner 1985, Döring et al. 2015, Pike 2014 |
| Disease frequency <i>B. cinerea</i> | Decrease | Biodyn | Danner 1985 |
| Winegrape quality | | | |
| Grape composition and wine quality | No difference | Biodyn | Danner 1985, Döring et al. 2015, Granato et al. 2015a, 2015b, Laghi et al. 2014, Meißner 2015, Parpinello et al. 2015, Partignani et al. 2017, Picone et al. 2016, Reeve et al. 2005, Tassoni et al. 2013, 2014 |
| Juice quality (by image forming methods) | Increase | Biodyn | Fritz et al. 2017 |
| γ -Aminobutyric acid, amino acids, organic acids, total phenols, total anthocyanins, <i>trans</i> -caffeic acid | Increase | Biodyn | Laghi et al. 2014, Picone et al. 2016, Reeve et al. 2005 |
| Juice acidity, sugars, alcohol content, phenolic compounds, wine color, total polymeric pigments, tannins, glutamine, coumaric acid, <i>trans</i> -caffeic acid | Decrease | Biodyn | Laghi et al. 2014, Meissner 2015, Parpinello et al. 2015, Picone et al. 2016 |
| Sensory characteristics | | | |
| Wine sensory characteristics, sensorial preference | No difference | Biodyn | Danner 1985, Martin and Rasmussen 2011, Parpinello et al. 2015, Partignani et al. 2017, Ross et al. 2009 |
| Sensorial preference of tasting panel (ranking) | Increase | Biodyn | Meißner 2015 |
| Costs | | | |
| Production costs | Increase | Biodyn | Danner 1985, Delmas et al. 2008 |
| Operational costs (undervine + canopy) | No difference | Biodyn | Santiago 2010 |

^aLAI, leaf area index; WUE, water use efficiency.

One hypothesis claims that biodiversity under organic management of perennial crops declines compared to conventional management. According to the intermediate disturbance hypothesis (Grime 1973, Horn 1975, Connell 1978, Bruggisser et al. 2010), biodiversity is linked to the level of disturbance in agroecosystems (caused by agricultural practices such as ploughing or mulching) in a nonlinear way. At intermediate disturbance levels the highest biodiversity is found. Perennial cropping systems are characterized by a lower level of background disturbance in relation to annual cropping systems. It is hypothesized that in perennial cropping systems, a further increase in disturbance, as caused by organic management, leads to a decline of biodiversity in contrast to annual cropping systems, where an increase of the level of background disturbance leads to an increase in biodiversity (Bruggisser et al. 2010).

Microbial Diversity in the Vineyard

Abundance of arbuscular mycorrhizal fungi increased under organic management (Freitas et al. 2011, Radić et al. 2014). Fungal endophyte colonization of the roots of grapevines and associated weeds under organic management, species richness, diversity indices, and arbuscular mycorrhizal spore abundance were higher compared to conventional management (Radić et al. 2014). No difference in fungal species richness was assessed in soils of biodynamically and conventionally managed vineyards in New Zealand (Morrison-Whittle et al. 2017). In contrast, management systems differed in the types of species present and in the abundance of single species. These results are supported by Hendgen et al. (2018), who recently observed a fungal community shift under organic viticulture in the topsoil layer without affecting fungal species richness in a long-term field trial in Germany. Bacterial biodiversity was increased in topsoil under organic management compared to conventional viticulture, the latter using mineral fertilizers, herbicides, and synthetic fungicides (Hendgen et al. 2018).

Several different vineyards in different locations differing in management approaches (organic or integrated) were compared concerning fungal endophytic communities on grapevine stems using both cultivation-based and cultivation-independent methods. The fungal endophytic communities under organic management were different from the ones under integrated management (Pancher et al. 2012). Fungicides used in the respective management approaches may be the driving force in shaping fungal community composition. The level of tolerance of the fungi to the applied fungicides is unknown.

The application of organic fertilizers in organic viticulture might be another factor that potentially influences fungal community composition. Variability of fungal endophytic communities from farms applying integrated pest management is described as smaller compared to organic farms. *Aureobasidium pullulans* was ubiquitous on farms with integrated pest management (Pancher et al. 2012). In contrast, *A. pullulans* was found to be characteristic for organically managed vineyards (Schmid et al. 2011). In this latter study, organically and conventionally managed vineyards were compared concerning

epiphytic and endophytic microbial communities on leaves, shoots, and grapes close to harvest in two subsequent years. Molecular analysis was performed by DNA extraction and fingerprinting. The conventional treatment showed highest abundance of *Sporidiobolus pararoseus*, whereas the organic treatment showed highest abundance of *A. pullulans*, as mentioned above. Schmid et al. (2011) explained *A. pullulans* presence in organic viticulture by its ability to metabolize inorganic S and absorb Cu. On the other hand, it remains unclear why Pancher et al. (2012) found *A. pullulans* to be characteristic for integrated pest management. Fungal ITS copy number was higher in organic compared to conventional treatment samples, indicating a higher fungal diversity in organic viticulture. Antiphytopathogenic potential of fungal isolates is described as higher for organic management. No differences concerning bacterial community composition were described (Schmid et al. 2011). The composition of the epiphytic microbial community on ripening Riesling grapes was not different among management systems in a field trial comprising integrated, organic, and biodynamic management (Kecskeméti et al. 2016).

Fungal species richness in bark and on ripe fruit assessed by metagenomics was higher in biodynamic compared to conventional viticulture (Morrison-Whittle et al. 2017). In bark, species richness differed, but not types of species or abundance. Species richness describes the number of species present in a certain environment, while abundance describes the number of single individuals of the same species present in a certain environment. While species richness and species abundance might be the same in two different environments, the types of species can potentially differ from one environment to the other. On ripe fruit, types of species and abundance differed between management systems. Differences in abundance of the genera *Columnosphaeria*, *Davidiella*, *Hanseniaspora*, *Chalara*, and *Trichothecium* were detected.

The observed differences in fungal biodiversity and abundance did not lead to a different community composition in the harvested juice (Sauvignon blanc) of biodynamically and conventionally managed vineyards (Morrison-Whittle et al. 2017). This might be due to a rough change of the environmental conditions from grape berries to pressed juice. Many yeasts that are present on harvested grapes are not adapted to the environment as it occurs in grape juice with low pH, lack of oxygen, and high sugar contents (Morrison-Whittle et al. 2017). Bagheri et al. (2015) assessed yeast population dynamics during spontaneous fermentation in Cabernet Sauvignon musts from biodynamic, integrated, and conventional management in South Africa. The farming systems differed in yeast community composition. The biodynamic vineyard had the highest culturable yeast diversity in both years of the study and the highest initial number of colony-forming units (cfu), indicating a higher species abundance. *Candida parapsilosis* and *Saccharomyces cerevisiae* were exclusively isolated from biodynamic musts at the start of fermentation. Cultivation-based assessment of the yeast community by Fourier transform infrared spectroscopy (FTIR), in contrast, showed a lower abundance of yeasts under biodynamic than conventional management (Guzzon et al. 2016). These

different results concerning yeast abundance under biodynamic and conventional viticulture might be due to different isolation and cultivation techniques and/or environmental conditions and management.

There is evidence that diversity of the microbial community on grapes and vines is enhanced under organic viticulture, although no characteristic fungi or bacteria for organic management could be found. Different results concerning the microbial community composition in one vineyard over the years indicate that the community composition is highly dependent on climatic conditions of every single vintage (Bagheri et al. 2015, Guzzon et al. 2016). Results concerning single yeast strains, fungi, or bacteria seem to be influenced by climatic conditions, sampling date, specific pest management strategies, or isolation techniques. The plant protection strategy is likely to highly influence yeast and fungal community composition, since most of the agents used in organic and conventional viticulture are fungicides against downy and powdery mildew. Little is known about their impact on the different yeast strains and fungi on grapes and vines.

Floral Biodiversity

Bruggisser et al. (2010) investigated biodiversity in organic and conventional Swiss vineyards at different trophic levels. Plant diversity, abundance, and richness were not enhanced under organic viticulture compared to conventionally managed vineyards, and no species were found that exclusively occurred in organically managed sites. Nascimbene et al. (2012) detected higher plant species richness in organically managed vineyards and adjacent noncrop areas compared to conventional management within an intensively used agricultural landscape in northern Italy. The positive effect of organic viticulture on local plant species richness could be due to the intensively farmed and homogeneous landscape in northern Italy compared to Switzerland, meaning that the landscape context might modify the beneficial effects of organic viticulture on biodiversity (Brittain et al. 2010, Nascimbene et al. 2012). The use of herbicides in conventional viticulture might account for the observed differences; mechanical operations and mowing regime did not differ between management systems (Nascimbene et al. 2012). In both vineyards and grassland strips, organic viticulture promoted growth of perennial species (higher abundance) in contrast to conventional farming, indicating a negative impact of herbicide application on the establishment of perennial plant species (Nascimbene et al. 2012). A recent study conducted in northern Spain comparing conventionally and organically managed vineyards found organic plots to host a richer community of vascular plants (Puig-Montserrat et al. 2017). Vegetation species density was higher under organic farming. As in the previous study, the use of herbicides might account for the lower community richness under conventional viticulture (Puig-Montserrat et al. 2017).

Earthworm Biodiversity

Earthworm abundance increased under organic and biodynamic management compared to integrated viticulture (Collins

et al. 2015b, Meißner 2015), and was even higher under biodynamic compared to organic viticulture in a replicated field trial in Germany (Meißner 2015). It is likely that the stimulation of the biological activity of the soil under organic and biodynamic management is due to the use of cover crop mixtures with a wide range of species, which enhances earthworm biodiversity and abundance. This effect was confirmed in different agroecosystems (Mäder et al. 2002, Blanchart et al. 2006). In contrast, Coll et al. (2011) found endogeic earthworm density and biomass to decrease under organic viticulture. Endogeic earthworms live in and feed on the soil and make horizontal burrows through the soil (Lee 1985). This result might suggest a shift in the earthworm community under organic management. The same study observed increased plant- and fungal-feeding nematode densities under organic management, as well as a decreased ratio of bacterial feeders/fungal feeders characteristic for organic farming (Coll et al. 2011).

Acarian Biodiversity

Populations of the predatory mite *Typhlodromus pyri*, a useful creature in vineyards for spider mite control (Duso 1989), have been shown to decrease under organic management because of the higher frequency of S sprays compared to conventional viticulture (Linder et al. 2006, Fleury and Fleury 2016). A study done in Australia, however, found abundance of predatory mites to be higher compared to conventionally managed blocks (Wheeler 2006). This could be due to a lower spraying frequency in Australia's drier conditions, or to the use of insecticides in the conventional plots. A comprehensive study in different Italian winegrowing regions that focused on predatory mite populations (Phytoseiidae and Tydeidae) in untreated, organic, and conventional vineyards found biodiversity in untreated and organic vineyards to be higher compared to conventional ones (Peverieri et al. 2009). Mite populations of untreated and organic vineyards were more similar to each other than conventional ones. This finding supports the hypothesis that arthropod biodiversity is increased under organic farming. Some predatory mite species were exclusively recorded in untreated and organic vineyards (*Kampimodromus aberrans* and *T. pyri*).

Biodiversity of Insects and Spiders in the Vineyard

Species richness of butterflies was enhanced under organic viticulture in northern Spain (Puig-Montserrat et al. 2017). The same authors investigated moth community composition in organic and conventional vineyards, but results were less significant. Conventional vineyard management is characterized by the use of wide-spectrum insecticides. Their use might affect the lepidopteran community and might therefore account for the loss of butterfly species richness under conventional viticulture. Larvae of moths are also susceptible to wide-spectrum insecticides. It remains unclear why the moth population was less affected in the latter study (Puig-Montserrat et al. 2017). Organic viticulture did not promote diversity or abundance of grasshoppers in a Swiss study (Bruggisser et al. 2010). Grasshopper diversity was even lower under organic compared to

conventional viticulture. Grasshopper diversity was in contrast enhanced by mulching compared to mowing, which induces a lower disturbance level. The authors concluded that the background disturbance level under organic viticulture in this case was too low to be beneficial for biodiversity of grasshoppers (Bruggisser et al. 2010).

Brittain et al. (2010) investigated whether isolated organic farms provided benefits for insect pollinators and pollination services in an intensively farmed landscape in northeast Italy. According to Brittain et al. (2010), organic and conventional vineyards did not differ in their floral resources or proportion of surrounding uncultivated land. Pollinator abundance, species richness, visitation rates of pollinators, and pollination of experimental potted plants were not affected by the management system. Vegetation control within rows did not differ between the two farming systems in this study and was done by mowing. Taking into account these two characteristics of the study, it is not surprising that no differences in pollinator population or pollination services were found.

When biodiversity of arthropods was compared for organic, biodynamic, and integrated viticulture in a field trial in Germany, the organic and the biodynamic treatment showed higher numbers of arthropods in the canopy and in the green cover, as well as an increased biodiversity of arthropods (Meißner 2015). Fleury and Fleury (2016) monitored ladybird populations in organically and conventionally managed vineyards in Switzerland and observed a higher abundance, but a lower biodiversity of ladybirds under organic management. Abundance of detritivores and Collembola was assessed in two Australian studies and shown to be higher under both organic and biodynamic management (Wheeler 2006, Collins et al. 2015b) than under conventional management.

Organic viticulture increased arthropod predator biodiversity and abundance in a study conducted in northwest Italy (Caprio et al. 2015). Different species responded differently to the different farming systems. Some carabids and spiders preferred organic, and some others preferred conventional vineyards. Preference patterns of spiders in general were shown not only to be driven by the farming system itself, but also by habitat features, such as grass cover, and small-scale landscape structures, such as bushes, trees, and small forest patches. Overall biodiversity and abundance of spiders such as arthropod predators were higher in organic vineyards and even in forest patches adjacent to organic vineyards, which were typically located below the sampled vineyards. Therefore, a leaching effect of chemicals and fertilizers could explain the enhanced biodiversity in organically managed sites, since no synthetic insecticides are allowed in organic viticulture (Caprio et al. 2015).

Another study on spider community composition under organic and conventional viticulture in northwest Italy confirmed the diversity of spider species to be higher in certified organic vineyards. The level of dominance of spider species was lower for certified organic than in conventional vineyards. A low level of dominance is one important parameter for indicating biodiversity, together with high species richness and high species abundance (Isaia et al. 2006).

Landscape heterogeneity seems to be important to maintain high diversification of spider hunting strategies, which may improve natural pest control (Isaia et al. 2006, Caprio et al. 2015). In the Swiss study assessing biodiversity of plants, grasshoppers, and spiders, no difference in spider abundance or diversity was detected between organic and conventional vineyards (Bruggisser et al. 2010). As shown previously, landscape features play a major role in determining diversity of spiders (Isaia et al. 2006, Caprio et al. 2015). In the case of Ligerz in northern Switzerland (Bruggisser et al. 2010), the diversity of landscape patterns and the proximity of a nature conservation area might modify the benefits of organic viticulture assessed in other studies (Brittain et al. 2010, Nascimbene et al. 2012).

Biodiversity of Birds in the Vineyard

Birds showed no significant response to treatments comparing organic and conventional vineyards in northern Spain (Puig-Montserrat et al. 2017), nor in northeast Italy (Assandri et al. 2016). Maintaining patches of residual habitats in the vineyard and enhancing landscape heterogeneity are two key factors to increase biodiversity of avian communities in vineyards in an intensively used agricultural landscape (Assandri et al. 2016). Mobile taxa such as birds may be less influenced by the management system of one specific vineyard (Puig-Montserrat et al. 2017). Nonetheless, Caprio and Rolando (2017) detected positive effects on the feeding ecology of great tits (*Parus major*) under organic viticulture in northwestern Italy. Landscape variables did not differ between organic and conventional vineyards. Differences in the number of nestlings fed per visit, and the weight of the nestlings, suggest that organic vineyards offer more feeding resources. The diet of nestlings was unaffected by the management system (Caprio and Rolando 2017).

Conclusions about Management Effects on Biodiversity

Pest management strategies, application of herbicides, the diversity of cover crops, and the use of compost seem to mainly influence biodiversity in the biosphere of vineyards. Plant species richness seemed to be higher under organic management, mostly due to the absence of herbicide application. Results were dependent on the landscape context, which might modify the beneficial effects of organic viticulture on biodiversity. Results concerning the earthworm population in different trials indicate an increase in abundance under organic and biodynamic viticulture, as well as a community shift. Cover crop mixtures rich in species used in organic and biodynamic, in contrast to conventional, viticulture could be responsible for the described phenomena. Results concerning predatory mite populations in vineyards are mixed and might be very dependent on the frequency of S sprays under organic viticulture. A comprehensive study from Italy (Peverieri et al. 2009) showed untreated and organic plots to have a higher biodiversity of predatory mites compared to the conventional treatment. Results concerning the biodiversity of different insect species are mixed. In general, abundance and diversity

of insects under organic viticulture either increased or did not differ from conventional viticulture. Results seemed to be highly dependent on the implementation of cover cropping in the organic treatment and on the landscape context. If investigated organic and conventional treatments did not differ in their flowering resources, it was unlikely that differences in insect biodiversity occurred. Some studies showed that fungicide and herbicide application in conventional viticulture had negative effects on insect biodiversity or abundance. Several studies showed the influence of landscape-induced background biodiversity in the region on biodiversity levels within the different management systems in vineyards. In intensively farmed and homogeneous landscapes, the enhanced biodiversity under organic viticulture was more evident, meaning that the landscape context might modify the beneficial effects of organic viticulture on biodiversity. The effect of the farming system seems to be more pronounced on less mobile taxa (Puig-Montserrat et al. 2017). Birds, for example, were little influenced by the management system.

The intermediate disturbance hypothesis (Grime 1973, Horn 1975, Connell 1978, Bruggisser et al. 2010) that predicted a loss of biodiversity in organically grown perennial crops due to the lower background disturbance level of organic farming must be rejected. Biodiversity in most trophic levels was enhanced under organic viticulture. Seventeen out of 24 studies showed a clear increase in biodiversity under organic viticulture. The impact of a decrease in disturbance does not only depend on the general level of disturbance, but also on the taxon investigated and the type of disturbance measured. The diversity maxima of different taxa may not be at the same position along the disturbance gradient. This is why the impact of a certain disturbance level on one taxon cannot necessarily be used to predict the impact on other taxa. Therefore, the intermediate disturbance hypothesis is only applicable for one single taxon, but not for a whole community of interacting taxa (Bruggisser et al. 2010). Organism-, site-, and crop-specific management strategies to enhance biodiversity in perennial crops should be developed. The conservation of specific taxa or organisms within an agricultural system is of high importance, because a general decrease in biodiversity within an agricultural system may lead to functional shifts when sets of species are replaced by others with different traits due to anthropogenic disturbance (Bruggisser et al. 2010).

Management Effects on Vine Growth

A reduction in vigor, expressed as pruning weight, shoot length, canopy density, or leaf area index (LAI) of organically managed vineyards compared to conventional management was observed for several white varieties such as Riesling, Kerner, and Müller-Thurgau (Hofmann 1991, Corvers 1994, Kauer 1994, Döring et al. 2015, Meißner 2015), and for the red varieties Grignolino and Cabernet Sauvignon (Malusà et al. 2004, Collins et al. 2015b, Pike 2015) (Table 2). However, LAI did not differ among treatments, according to Corvers (1994) (Riesling and Kerner). When pruning weight of organic and conventional management was compared by meta-regression

analysis, taking into consideration all available data sets of scientific trials, organic and biodynamic treatments showed 21% less growth as pruning weight compared to conventional treatments (Figure 1).

Pruning weight of organic and biodynamic treatments differed from conventional/integrated treatments in the respective field trials. The environmental factors had a significant influence on the pruning weight, but no interactions between treatment and environment were observed, meaning that organic and biodynamic treatments always showed lower pruning weights regardless of the location of the trial (Supplemental Table 2). All the studies included showed an average reduction in pruning weight under organic management (Supplemental Figure 1).

The relative vegetative growth expressed as pruning weight under organic, in comparison to conventional, viticulture ranged between 57.1% and 104%. No clear relationship between the level of conventional pruning weights and relative organic pruning weights could be observed, taking into account the data of field experiments available (Figure 2).

Chlorophyll content and macronutrient supply in leaves at veraison did not show differences among treatments (Linder et al. 2006, Döring et al. 2015, Meißner 2015). Danner (1985) reported a reduction of the nutrient supply for organic production in a field trial comparing organic, biodynamic, and conventional viticulture from 1979 to 1983 in Mailberg, Austria (Grüner Veltliner). N content in leaves was lower under organic and biodynamic management (Danner 1985, Malusà et al. 2004). Döring et al. (2015) showed N content in leaves at veraison under organic and biodynamic management to be higher in comparison to integrated management. At the same time, mineralized N content in the soil of the respective

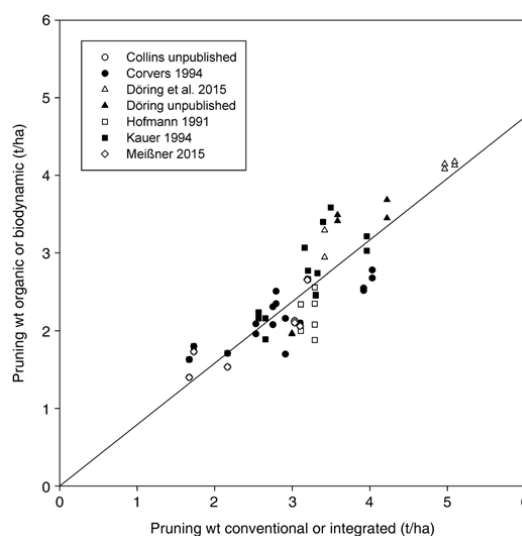


Figure 1 Growth expressed as pruning weight of conventional or integrated vineyards versus organic or biodynamic vineyards ($y = 0.7921x$; $R^2 = 0.74$; $n = 56$).

treatments was higher, as mentioned above. In the same trial, chlorophyll content was shown to decrease at veraison under organic and biodynamic management (Döring et al. 2015, Meißner 2015). Magnesium (Mg) and P content in leaves decreased under organic and biodynamic production systems compared to conventional systems in Germany and Australia (Collins et al. 2015b, Döring et al. 2015) (Table 2).

Macronutrient supply and content in leaves seems to be highly influenced by the management within organic, biodynamic, or conventional treatments. It was shown that it is possible to ensure N and macronutrient supply without the use of synthetic N fertilizers (Wheeler 2006, Probst et al. 2008, Coll et al. 2011, Collins et al. 2015b, Döring et al. 2015, Meißner 2015), although N content in the soil and in the leaves is highly dependent on fertilization strategy and water availability. It is notable that P content in the Australian and the German long-term field trial was lower under biodynamic management, and in Australia, also under organic management. This could be due to the lower water availability in the respective systems measured as soil moisture (Collins et al. 2015b) or predawn water potential (Ψ_{pd}) (Döring et al. 2015). Mg content in leaves or petioles was also shown to be lower under organic and biodynamic management in both trials and might account for the decrease in chlorophyll content at veraison monitored in the organic and the biodynamic plots in the long-term trial in Germany.

Physiological performance was reported to decrease under organic and biodynamic management (Döring et al. 2015). In case of the biodynamic production system, Ψ_{pd} also decreased in comparison to the conventional production system in Germany, whereas no differences in stem water potential among systems could be detected close to harvest in Australia (Döring et al. 2015) (Collins and Döring, unpublished) (Table 2).

Growth and vigor expressed as pruning weight, shoot length, canopy density, or LAI decreased under organic and

biodynamic, in comparison to conventional, viticulture. Since in organic plots, microbial soil activity and soil organic carbon were generally higher and no consistent difference in soil N, P, or S could be observed in several field trials, these parameters cannot account for the observed differences in growth. The only study that reports a reduction in nutrient supply under organic production was performed in Austria at the beginning of the 1980s. It seems more likely that the observed reduction in physiological performance in organic plots reported by Döring et al. (2015) might account for the growth differences between conventional and organic management. The authors observed changes in physiological performance under organic and biodynamic management after full bloom, especially under dry conditions in Germany. At the same time, Ψ_{pd} decreased under organic and biodynamic management. It could be hypothesized that the cover crop mixture rich in legumes used in organic and biodynamic viticulture to enhance biodiversity and to ensure N supply has an impact on water availability in the soil, and thus competes with the root system of the vines. Under dry conditions with irrigation in Australia, no differences in stem water potential among treatments could be observed before harvest (Collins and Döring, unpublished), although growth and canopy density in the organic and biodynamic plots decreased in the trial. This could be due to the fact that natural vegetation between rows occurs in the organic and the biodynamic system in spring when soil water availability is higher. During the dry growing season, the natural vegetation senesces, but it could still influence root growth in the respective management systems. When soil moisture was assessed in the long-term field trial in Australia during the growing season 2010 to 2011, a significant decrease of soil moisture content under organic management at 20 cm up to 1 m depth was observed compared to the high input conventional system (Collins et al. 2015b). It is likely that differences in the root system of the vines or the water availability in the soil due to cover cropping might account for different levels of plant growth regulators such as abscisic acid and cytokinin that strongly determine growth and vigor (Stoll et al. 2000).

When comparing yield/pruning weight ratios of organic and biodynamic viticulture, they were found to be significantly lower in biodynamic viticulture (Merlot) (Reeve et al. 2005) (Table 3). This difference was due to a slightly higher yield in the organic treatment, while pruning weights themselves did not differ between treatments. However, other studies did not assess differences in the yield/pruning weight ratios between organic and biodynamic plots (Collins et al. 2015b, Döring et al. 2015). No differences between organic and biodynamic treatments were observed concerning pruning weight, LAI, or leaf area-to-fruit weight ratio (Döring et al. 2015, Meißner 2015). The ratio of yield:pruning weight was significantly lower under biodynamic viticulture (Reeve et al. 2005), but the other studies showed ratios of pruning weight under organic and biodynamic management to be similar (Collins et al. 2015b, Döring et al. 2015, Meißner 2015).

Macronutrients and chlorophyll content in leaves did not differ between organic and biodynamic plots for Riesling

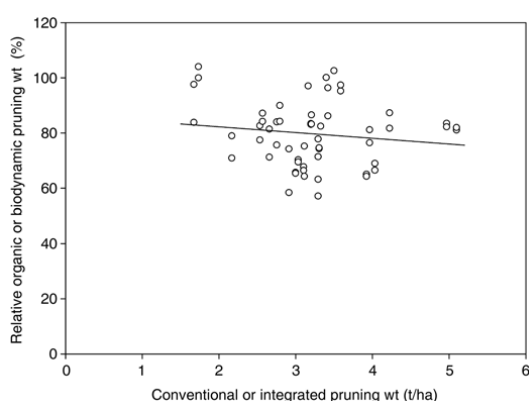


Figure 2 The relative growth expressed as pruning weight of organic or biodynamic viticulture as a function of the absolute conventional or integrated growth expressed as pruning weight from field experiments ($y = -2.0791x + 86.389$; $R^2 = 0.0201$; $n = 56$).

(Döring et al. 2015, Meißner 2015) (Table 3). Since all nutrients are transported within the plant in the xylem, and xylem sap flow can be ensured only if there is enough plant-available soil water, nutrient supply and water uptake are closely related (Yang and Tyree 1992). Two recent studies on organic and biodynamic viticulture observed significantly lower Ψ_{pd} in the biodynamic plots, cv. Riesling in Germany (Döring et al. 2015) and cv. Sangiovese in Italy (Botelho et al. 2016). The relation of predawn or soil water potential and stomatal conductance of plants is usually plant- and environment-specific, but nonetheless very close (Tramontini et al. 2014). One of the two studies that observed differences in Ψ_{pd} between organic and biodynamic viticulture observed stomatal conductance of the biodynamic plots to be lower (Botelho et al. 2016), whereas in the other study, the lower water potential did not have implications on the physiological performance of the plants (Döring et al. 2015). In the Italian field trial where lower stomatal conductance under biodynamic viticulture occurred, a significant increase in leaf enzymatic activity (endochitinase, β -N-acetylhexoaminidase, chitin 1,4- β -chitobiosidase, β -1,3-glucanase) of the biodynamic plots for Sangiovese was observed (Botelho et al. 2016). The enzymatic activities that were found to increase under biodynamic management are linked to biotic and abiotic stress, and are associated with induced resistance against several fungi such as powdery mildew (*Erysiphe necator*), downy mildew (*P. viticola*), and Botrytis bunch rot (*Botrytis cinerea*) (Giannakis et al. 1998, Reuveni et al. 2001, Magnin-Robert et al. 2007). One hypothesis is that especially the biodynamic horn silica preparation 501 (Table 1) made from quartz powder and used in very small quantities might upregulate plant defense mechanisms attributed to induced plant resistance (Botelho et al. 2016). However, the plots of the study by Botelho et al. (2016) were replicated, but not randomized. This is why the observed changes in physiological performance and enzymatic activity cannot clearly be attributed to the treatment and need to be confirmed.

Management Effects on Yield

A meta-analysis on annual and perennial crops under organic and conventional management reveals organic yields of individual crops to be on average 80% of conventional yields (De Ponti et al. 2012). This organic yield gap, however, may differ among crops and regions. It is hypothesized that the yield gap between organic and conventional production is higher than 20% at high yield levels and lower than 20% at low yield levels (De Ponti et al. 2012). It is hypothesized that the increasing yield gap with higher conventional yield levels may be due to yield losses by pests and diseases and/or lower P availability under organic farming. The average relative yield for organically grown fruits was 72%, including grapes, melon, apricot, black currant, cherry, peach, pear, and others (De Ponti et al. 2012). Another meta-analysis found yields of organically grown annual crops and animal products such as milk to be 91% of the conventional yields (Stanhill 1990).

A yield loss from 10% up to 30% is reported for organic viticulture, compared to conventional production for several

white varieties such as Riesling, Kerner, Müller-Thurgau, Grüner Veltliner, Chardonnay, and Seyval, and for red varieties Grignolino, Cabernet Sauvignon, Merlot, Shiraz, and Concord (Danner 1985, Hofmann 1991, Corvers 1994, Kauer 1994, Pool and Robinson 1995, Malusà et al. 2004, Wheeler 2006, Collins et al. 2015a, Döring et al. 2015, Meißner 2015) (Table 2). Meta-regression analysis shows that organic and biodynamic treatments have on average 18% less yield compared to conventional treatments (Figure 3). The average yield gap observed in viticulture is similar to that in annual crops.

Yield of organic and biodynamic treatments differed from conventional/integrated treatments in the respective field trials. The environmental factors had a significant influence on the yield levels, but no interactions between treatment and environment were observed, meaning that organic and biodynamic treatments always showed significantly lower yields regardless of the location of the trial (Supplemental Table 2). All the studies included in the meta-analysis and the meta-regression showed lower average yields for organic or biodynamic, compared to conventional, management (Supplemental Figure 2).

The yield gap under organic compared to conventional viticulture ranges from 44.2% to 119.4% for the data included in the meta-analysis. Looking at the relative organic yield in proportion to the yield level of the conventional or integrated counterpart, no clear relationship between conventional yield level and relative organic yield can be observed (Figure 4), meaning that organic relative yields do not automatically decrease when yield levels increase in conventional viticulture.

In the case of Chasselas in Perroy (Waadt, Switzerland) (Linder et al. 2006), Riesling in Geisenheim (Rheingau, Germany) (Döring et al. 2015, Meißner 2015), and Elvira in Geneva (New York, US) (Pool and Robinson 1995), a reduction

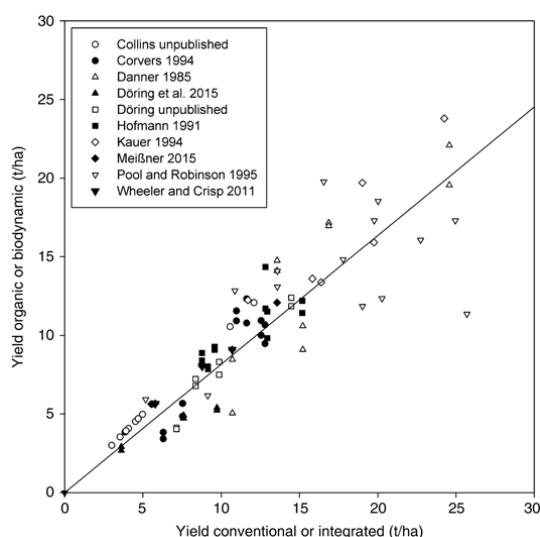


Figure 3 Yield of conventional and integrated vineyards versus organic and biodynamic managed vineyards ($y = 0.8184x$; $R^2 = 0.80$; $n = 92$).

of the berry weight under organic and biodynamic production was observed. In contrast, single berry weight did not differ among treatments from 1990 to 1992 in Erbach and Hattenheim (Rheingau, Germany) (Riesling and Kerner) (Corvers 1994), and from 1990 to 1994 in Geneva (New York, US) (Concord and Seyval) (Pool and Robinson 1995) (Table 2).

Organic and biodynamic plots showed lower compactness of bunches (Riesling) (Döring et al. 2015, Meißner 2015). The number of berries/bunch and the average bunch weight were significantly lower under organic and biodynamic management in the Corvers (1994) and Döring et al. (2015) studies, but Pool and Robinson (1995) did not report a difference in the number of berries/bunch and the average bunch weight between management systems (Table 2). The reduced berry weights, reduced bunch weights, and reduced number of berries/bunch that were observed under organic and biodynamic management in many trials could be due to different water availability in the soil (Collins et al. 2015b) and a reduced physiological performance around full bloom (Döring et al. 2015); these could be reasons for the yield decrease described above. Fruit set was not assessed in any of the trials, but fruit set is likely to differ between treatments, because the treatments were shown to differ in growth, water availability in the soil, and physiological performance at full bloom.

Döring et al. (2015) provided evidence that the vine water status, and thus the physiological performance, differ between organic or biodynamic and conventional viticulture. Since reproductive development of *Vitis vinifera* is highly sensitive to the water and N status, the lower water availability in the organic and the biodynamic management system might account for the differences in physiological performance and might cause yield differences. Lower water availability early in the season was shown to cause decreases in yield and cluster weight (Matthews and Anderson 1989). Since the period from initiation to maturation of winegrapes comprises two growing seasons, early season water deficit might have implications for cluster weight of the current year and the number

of clusters of the subsequent year (Matthews and Anderson 1989, Döring et al. 2015). There is evidence that water availability in hot as well as in cool climate viticulture plays a key role in determining vigor and yield under organic and biodynamic viticulture. How P and Mg availability are influenced by the different water availability in the different viticultural management systems should be a subject of further research. It is still unclear to what extent the lower P and Mg availability under organic and biodynamic viticulture determines growth and yield of the respective systems.

No yield differences were observed between organic and biodynamic treatments for Merlot, Sangiovese, Cabernet Sauvignon, and Riesling (Reeve et al. 2005, Collins et al. 2015a, Döring et al. 2015, Meißner 2015, Botelho et al. 2016) (Table 3). Danner (1985) detected lower yields for the biodynamic plots than for the organic plots in two out of five years of the study done in Austria on Grüner Veltliner. The organic and the biodynamic treatments did not differ in the number of bunches/vine, cluster weight, cluster compactness, or berry weight (Reeve et al. 2005, Döring et al. 2015, Meißner 2015).

Management Effects on Disease Incidence

Disease incidence of downy mildew (*P. viticola*) did not show any differences comparing organic, biodynamic, and conventional production for Grüner Veltliner in Austria under higher disease pressure, and for Cabernet Sauvignon in Australia under low disease pressure (Danner 1985, Pike 2015). For Riesling under more humid climatic conditions, the incidence of downy mildew was significantly higher under organic and biodynamic production when the disease occurred (Döring et al. 2015) (Table 2). For Chasselas in Switzerland, the organic treatment showed significantly higher disease incidence of powdery mildew (*E. necator*) (Linder et al. 2006). Results are mixed concerning the disease incidence of Botrytis bunch rot (*B. cinerea*). Danner (1985) reported that the organic treatment showed a higher disease incidence of Botrytis bunch rot compared to the biodynamic and the conventional treatment in three out of five years, whereas the biodynamic treatment showed a higher disease incidence of Botrytis bunch rot in just one out of five years, compared to the conventional system. For Riesling, the organic and biodynamic plots showed lower disease incidence of Botrytis bunch rot compared to the integrated plot from 2006 to 2009 (Meißner 2015). The field trial was managed and conducted in the same way after conversion. In the following years of the same field trial from 2010 to 2012, the biodynamic treatment showed significantly higher disease incidence of Botrytis bunch rot compared to the integrated management system, whereas the organic treatment did not differ from the integrated plots (Döring et al. 2015) (Table 2). Under dry conditions in Australia, the organic and the biodynamic plots did not differ from the conventional plots with respect to disease incidence of Botrytis (Pike 2015). This might be due to the low disease pressure under Australian conditions and Cabernet Sauvignon bunch architecture preventing infections of Botrytis bunch rot (Table 2). The organic and the biodynamic system showed significantly less sour rot on bunches in the

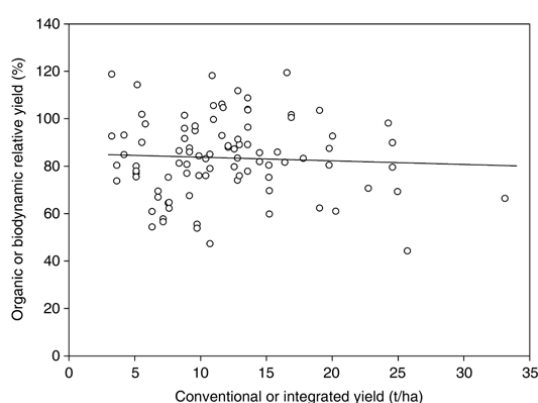


Figure 4 The relative yield of organic or biodynamic viticulture as a function of the absolute conventional or integrated yield from field experiments ($y = -0.1526x + 85.348$; $R^2 = 0.003$; $n = 92$).

field trial in Geisenheim (Rheingau, Germany) (Döring et al. 2015, Meißner 2015).

The direct comparison of biodynamic versus organic viticulture revealed a higher disease incidence of *Botrytis* under organic management in three out of five years in the field trial in Austria (Danner 1985), but no difference was detected in the field trial in Germany (Döring et al. 2015) or in Australia (Pike 2015) (Table 3). Organic and biodynamic viticulture did not differ in the disease incidence of downy mildew (Danner 1985, Döring et al. 2015, Pike 2015).

Disease incidence seems to be highly dependent on the grapevine variety, the location and its microclimate, the vineyard management, and the environmental conditions of each year. Still, it is not surprising that disease incidence of some grapevine diseases such as powdery and downy mildew is higher under organic or biodynamic management compared to conventional viticulture, since the organic and the biodynamic systems exclusively rely on fungicides such as Cu and S and on plant resistance improvers. All these agents are strictly protectants and they do not act curatively, as some synthetic fungicides do. Besides that, no botryticides are applied in organic or biodynamic viticulture. Döring et al. (2015) quantified the potential yield loss in organic and biodynamic viticulture due to downy mildew over a three-year-period and concluded that only up to 10% of the observed yield reduction in organic and biodynamic management could be attributed to the infestation with downy mildew in the year with the most severe attack of downy mildew. This clearly underlines that other mechanisms must play a key role in causing the yield gap between organic and conventional production.

Management Effects on Fruit, Juice Composition, and Wine Quality

The impact of organic viticulture on grape quality parameters, juice, and wine quality is inconclusive (Table 2). In a number of trials, no consistent differences were observed in grape composition of several researched grape varieties (Danner 1985, Hofmann 1991, Kauer 1994, Henick-Kling 1995, Malusà et al. 2004, Linder et al. 2006, Tassoni et al. 2013, 2014, Collins et al. 2015a, 2015b, Döring et al. 2015), whereas Hofmann (1991) reported differences in winegrape quality between organic and conventional production, depending on plant protection strategy and incidence of *Botrytis* at harvest. Coffey (2010) detected higher levels of zinc and iron (Fe) in berries from organic management (Cabernet Sauvignon) in Australia. Corvers (1994) and Meißner (2015) observed that the integrated treatment showed significantly higher must acidity for the varieties Riesling and Kerner, in Germany, whereas in a study in Italy, organic Sangiovese wines had higher malic acid and volatile acidity (Beni and Rossi 2009). Tobolková et al. (2014) detected higher citric acid in Slovakian white wines from conventional management. No differences in juice and wine quality were observed, including acidity, macronutrients, and phenolic compounds (Danner 1985, Kauer 1994, Henick-Kling 1995, Dupin et al. 2000, Granato et al. 2015b, 2015c, 2016, Meißner 2015) (Table 2). Fritz et al. (2017) assessed juice quality in the first year of

conversion according to image-forming methods (biocrystallization, capillary dynamolysis, and circular chromatography image analysis [Huber et al. 2010, Zalecka et al. 2010]), and ranked grape juices from organic and biodynamic plots better than grape juices from integrated plots due to their strength of form expression and their resistance to deterioration (Riesling). Cozzolino et al. (2009) correctly classified 85% of their samples of Australian organic and nonorganic wines according to mid-infrared spectra by discriminant partial least squares. Meta-regression analysis shows that the juice sugar concentration of organically and biodynamically managed vineyards was almost the same as that of conventionally managed vineyards (Figure 5).

TSS in juice of organic and biodynamic treatments did not differ from conventional/integrated treatments in the respective field trials. The geographic location of the trials had a significant influence on levels of TSS, but no interactions between treatment and environment were observed, meaning that organic and biodynamic treatments never differed from conventional treatments in TSS in juice regardless of the location (Supplemental Table 2). All the studies included in the meta-analysis and meta-regression showed similar amounts of TSS for organic and conventional management (Supplemental Figure 3).

It was shown that growth and yield of grapevines under organic and biodynamic management generally decrease. One very important parameter determining potential level of TSS in grape juice is the leaf area-to-fruit weight ratio (Kliewer and Dokoozlian 2005). Döring et al. (2015) measured this ratio under integrated, organic, and biodynamic management.

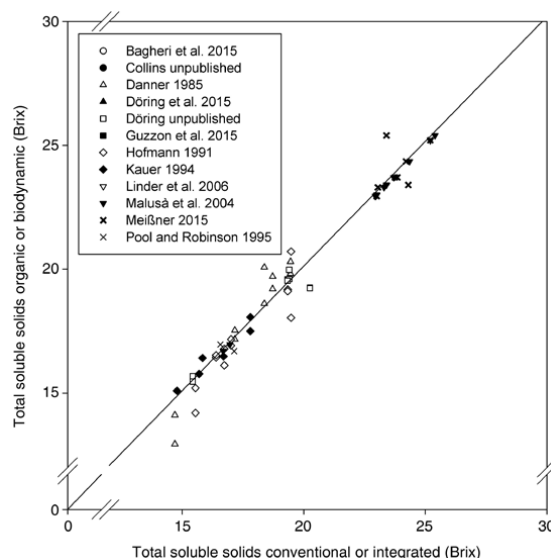


Figure 5 Juice sugar concentration of conventionally or integrated vineyards versus organically or biodynamically managed vineyards ($y = 1.0068x$; $R^2 = 0.96$; $n = 85$).

The organic and the biodynamic treatments showed slightly higher levels of leaf area-to-fruit weight ratio, but there was no difference among treatments. One reason why organically and biodynamically managed vineyards do not differ from conventional vineyards in TSS in juice could be the simultaneous decrease of growth and yield, resulting in a similar ratio of leaf area to fruit weight. Results by Collins et al. (2015b) concerning the ratio of yield-to-pruning weight support that there is no difference among the systems concerning the ratio of reproductive and vegetative growth. Another reason for the fact that systems did not differ in the amount of TSS at harvest might be that physiological performance (assimilation rate, transpiration rate, and stomatal conductance) after veraison, which highly determines final sugar content and berry quality traits, did not differ among treatments when measured in the long-term field trial in Germany (Hardie and Considine 1976, Döring et al. 2015).

Yeast-available N content (N-OPA) was shown to increase under biodynamic management after conversion (Döring et al. 2015), but N-OPA content did not differ among treatments in the first years of the same trial (Meißner 2015). The application of systemic fungicides in the integrated plot (Oliva et al. 2011) and the lower content of N in the soil of the integrated plots together with the high yields may have caused the decrease in N-OPA levels in berries of the integrated treatment (Döring et al. 2015).

Yildirim et al. (2007) found putrescine content to be significantly higher under organic viticulture, while Tassoni et al. (2013) did not detect differences in the content of biogenic amines in wines from different management systems.

There is evidence that anthocyanin and flavonoid content in berry skin; polyphenol content, antioxidant potential, and phenolic acid content in juice and wine; and resveratrol content and enzyme polyphenol oxidase concentration in grapes increase under organic management (Tintunen and Lehtonen 2001, Micelli et al. 2003, Malusà et al. 2004, Yildirim et al. 2004, Núñez-Delgado et al. 2005, Otreba et al. 2006, Dani et al. 2007, Vrček et al. 2011, Rodrigues et al. 2012, Buchner et al. 2014, Granato et al. 2015a). Other studies did not observe any differences in the polyphenol or anthocyanin profiles of grapes and wines, their carotenoid and *trans*-resveratrol content, content of *p*-coumaric acid, or their antioxidant activity (Lante et al. 2004, Mulero et al. 2009, 2010, Bunea et al. 2012, Tassoni et al. 2013, Collins et al. 2015a, 2015b, Garaguso and Nardini 2015). Total polyphenol content and antioxidant activity in wines even decreased under organic viticulture in some other studies (Yildirim et al. 2004, Beni and Rossi 2009). Moreover, ascorbic acid equivalents, ferric-reducing power, and Cu and Fe in wines were found to be reduced in organic viticulture (Tobolková et al. 2014).

It is likely that the different treatments produced different polyphenol contents, since the synthesis of these parameters is highly linked to light interception in the canopy. Organic and biodynamic treatments showed significantly lower growth, lower canopy density, and lower secondary shoot growth. This lower vigor might induce higher levels of flavonoids and anthocyanins, and thus a higher antioxidant

potential (Cortell et al. 2005). On the other hand, the organic and the biodynamic plots also had lower yields, which might result in no change in the polyphenol content. Fruit-zone leaf removal potentially has a strong effect on light interception, and thus on phenolic composition of the grapes. In the trials comparing the different management systems, fruit-zone leaf removal was not implemented so as not to interfere too much with the systems' performance.

Several studies focused on grape composition under organic and biodynamic viticulture, including TSS, acidity, macronutrients, and phenolic compounds. Most of the studies revealed that there was no difference for varieties such as Grüner Veltliner, Merlot, Pignoletto, Sangiovese, Cabernet Sauvignon, Albana, Lambrusco, and Riesling (Danner 1985, Reeve et al. 2005, Tassoni et al. 2013, 2014, Laghi et al. 2014, Collins et al. 2015b, Döring et al. 2015, Granato et al. 2015a, 2015c, Meißner 2015, Parpinello et al. 2015, Picone et al. 2016, Patrignani et al. 2017) (Table 3). Almost all the studies included in the meta-analysis and meta-regression showed similar levels of TSS for organic and biodynamic viticulture.

Nonetheless, some authors assessed differences in the chemical composition of berries, juices, or wines grown organically and biodynamically (Table 3). Meißner (2015) detected a lower juice acidity in fruit managed biodynamically (Riesling). Fritz et al. (2017) assessed juice quality in the first year of conversion according to image-forming methods (biocrystallization, capillary dynamolysis, and circular chromatography image analysis [Huber et al. 2010, Zalecka et al. 2010]), and ranked grape juices from biodynamic plots better than grape juices from organic plots (Riesling). Some studies revealed that there was an increase in total phenols, total anthocyanins, and γ -aminobutyric acid, as well as amino acids and organic acids under biodynamic viticulture for Sangiovese and Merlot (Reeve et al. 2005, Laghi et al. 2014, Picone et al. 2016). Laghi et al. (2014), Parpinello et al. (2015), and Picone et al. (2016) detected a decrease in sugars, alcohol content, phenolic compounds, wine color, total polymeric pigments, and tannins, as well as coumaric and *trans*-caffeic acid (cv. Sangiovese). One hypothesis could be that the lower stomatal conductance, as observed in biodynamically managed plants, led to a higher concentration of internal CO₂ (Botelho et al. 2016, Picone et al. 2016). A higher internal CO₂ concentration could then lead to a predominance of the anaerobic metabolism in biodynamically grown berries compared to organically grown berries (Picone et al. 2016). It is thought that in berries of biodynamic management, the fermentative pathway is activated (Picone et al. 2016). Lower sugar concentration and an increased concentration of organic acids such as lactate and malate in biodynamically grown berries might be signs of the activation of the anaerobic metabolism (Picone et al. 2016), although further research is needed to confirm this hypothesis. Moreover, the field trial on Sangiovese had no randomized field replicates (Laghi et al. 2014, Parpinello et al. 2015, Botelho et al. 2016, Picone et al. 2016). This is why it is not clear whether the observed phenomena are an effect of the plot or of the treatment.

Results by Meißner (2015) suggested a lower juice acidity in biodynamically grown grapes, which is contrary to a higher concentration of organic acids, as found by Picone et al. (2016). The increase of phenolic compounds under biodynamic management, as described by Reeve et al. (2005), could confirm the hypothesis of an upregulation of substances attributed to induced resistance in biodynamically grown plants, as expressed by Botelho et al. (2016). Still, Parpinello et al. (2015) found that total polymeric pigments, tannin concentration, and total color under biodynamic management decreased in the first two years after conversion, but again this field trial did not have randomized replicates.

Management Effects on Fruit and Wine Sensory Characteristics

Berries derived from the long-term trial in Australia on Cabernet Sauvignon were submitted to berry sensory analysis in order to assess grape sensory pulp properties. Berries from organic management resulted in having a significantly higher pulp juiciness compared to berries from the conventional treatment in the third year of conversion (Coffey 2010).

Results concerning the sensory characteristics of wines derived from organic and conventional management are heterogeneous. Wines derived from several field trials revealed no influence of management on wine sensory characteristics when rank sum tests were applied (Danner 1985, Kauer 1994, Meißner 2015). Martin and Rasmussen (2011) compared pairs of organically and conventionally grown wines that differed in their total polyphenol concentration, but found no difference in the sensory characteristics. Dupin et al. (2000) compared commercially available wines of organic and conventional production and did not observe differences in the wine sensory attributes. However, in two of these studies, the wines from conventional management were perceived as more floral, fruity, vegetal, and complex (Dupin et al. 2000, Meißner 2015), whereas the wines from biodynamic management tended to be more balanced and full-bodied, with a stronger minerality and more length (Meißner 2015). In this study, wines from biodynamic management were preferred by the tasting panels in rank sum tests (Meißner 2015). Wines from the Australian long-term-trial on Cabernet Sauvignon were characterized by quantitative descriptive analysis, and wines from organic and biodynamic plots were assessed as more rich, textural, complex, and vibrant in comparison to wines from conventionally managed plots (Collins et al. 2015a). Henick-Kling (1995) found wines from organic management (Seyval) to be significantly more spicy and less skunky compared to conventional wines, and panelists preferred the wine from organic plots. Organically grown Sangiovese wines from an Italian field trial were described as less astringent with a higher overall acceptance by the sensory panel (Beni and Rossi 2009). Trebbiano wines from the same trial were described as unbalanced and acidic with respect to the organic product (Beni and Rossi 2009).

No differences could be detected in sensory characteristics of the wines between organic and biodynamic management for Grüner Veltliner and Sangiovese (Danner 1985, Collins

et al. 2015a, Parpinello et al. 2015, Patrignani et al. 2017). By contrast, Meißner (2015) reported a sensorial preference of Riesling wines from biodynamic management in comparison to the ones from the organic plots. Ross et al. (2009) detected differences between Merlot wines from organic and biodynamic plots of a field trial in two out of four years, but sensory characteristics attributed to the different wines were not consistent over the years.

Management Effects on Production Costs and Efficiency

The increase in production costs for organic and biodynamic viticulture assessed in Europe, the US, and Australia ranged between 7 and 90% compared to conventional production, although the increase in costs was highly dependent on the size of the winery and the timespan since conversion (Danner 1985, White 1995, Linder et al. 2006, Delmas et al. 2008, Santiago 2010, Santiago and Johnston 2011, Collins et al. 2015b). The increase in production costs was mainly due to yield reduction and higher costs for under-vine weed control and compost management, whereas costs for irrigation and canopy management decreased (Santiago 2010, Santiago and Johnston 2011). Wheeler (2006) found input costs as well as labor input costs of organic viticulture to be higher compared to conventional viticulture. In the long-term field trial in Australia, organic and biodynamic viticulture produced 74% and 65%, respectively, of the gross margins compared to high-input conventional viticulture (Collins et al. 2015b). Guesmi et al. (2012) investigated the productive efficiency of organic and conventional wineries in Catalonia and found organic farms to have higher efficiency ratings than conventional farms in the area, mostly due to improved agricultural performance, better management of their inputs, and organic price premiums. Biodynamic viticulture in a Spanish study showed substantially lower environmental burdens compared to conventional viticulture determined by life cycle assessment (Villanueva-Rey et al. 2014).

The life cycle assessment in this case compares all inputs and outputs (trellises, fertilizers, pesticides, energy, water, field operations, and emissions) for a production system, e.g., for producing a certain amount of grapes. It evaluates their environmental impact for different forms of viticulture (Villanueva-Rey et al. 2014). Kavargiris et al. (2009) found total energy inputs, fertilizer and plant protection products application, fuel inputs, and greenhouse gas emissions to be higher in conventional compared to organic wineries of the same size in Greece. On the other hand, grape yield, pomace, and ethanol from pomace were also higher in conventional wineries (Kavargiris et al. 2009). According to Delmas et al. (2008), costs for biodynamic grapegrowing are between 10 and 15% higher than for organic grapegrowing. Santiago (2010) found biodynamic wineries in Australia to have only 7% higher operational costs, including canopy and under-vine management costs, than organic wineries. The same study highlighted that large biodynamic wineries had lower operational costs/ha compared to organic wineries, in some cases, even lower operational costs/ha compared to conventional

wineries (Santiago 2010). Overall costs for winemaking are similar for conventional, organic, and biodynamic wine (Delmas et al. 2008).

Conclusions

Stimulation of soil nutrient cycling by compost application, the implementation of cover crop mixtures with a wide range of species, and denial of mineral fertilizers and herbicides, as practiced in organic and biodynamic viticulture, take some years to make an impact on N levels and on microbial activity in the soil. This is why long-term field trials seem to be crucial for a better understanding of the management systems. The contribution of soil microbial communities to soil fertility and the consequences on plant growth, especially in comparison to mineral fertilization, are little understood and need more scientific attention to characterize the underlying phenomena.

Biodiversity at different trophic levels was enhanced under organic and biodynamic viticulture compared to conventional management. Seventeen out of 24 studies showed a clear increase in biodiversity under organic and biodynamic viticulture. Pest management strategies, herbicide application, addition of compost, and diversity of cover crops seem to mainly influence biodiversity in the biosphere of vineyards. The contribution of an enhanced biodiversity to abundance and biodiversity of antagonistic insects in the vineyard should be further investigated and quantified.

Growth of vines expressed as pruning weight under organic and biodynamic viticulture decreased by 21%, although single study outcomes were heterogenic. This might be due to different soil water availability in organic viticulture, which might result in a lower physiological performance, especially after full bloom. It is likely that differences in the root systems of the vines or the water availability in the soil due to cover cropping might account for different levels of plant growth regulators, such as gibberellic acid, cytokinin, and auxin, which strongly determine growth and vigor. The mechanisms that influence growth in organic and biodynamic viticulture should be further investigated by assessing hydraulic conductivity, stomatal conductance, and phytohormone contents at the same time.

A yield decrease of 18% in organic and biodynamic viticulture compared to conventional viticulture was observed when all available data from scientific field trials were assessed. Since reproductive development of *V. vinifera* is highly sensitive to water status, the lower water availability and the lower physiological performance after full bloom in the organic and the biodynamic management systems might cause yield differences. Since the period from initiation to maturation of winegrapes comprises two growing seasons, early season water deficit might have implications for cluster weight of the current year and the number of clusters of the subsequent year. More information about the influence of differing soil moisture content and physiological performance of the management systems on fruit set should be gained in the future.

Treatments did not differ in TSS in juice. It was shown that growth and yield of grapevines under organic and biodynamic

management generally decrease. One very important parameter determining potential levels of TSS in grape juice is the leaf area-to-fruit weight ratio. One reason why organically and biodynamically managed vineyards do not differ from conventional vineyards in TSS in juice could be the simultaneous decrease of growth and yield that results in a similar ratio of leaf area to fruit weight.

Organic and biodynamic treatments showed significantly lower growth, lower canopy density, and lower secondary shoot growth. This lower vigor might induce higher levels of flavonoids and anthocyanins, and thus a higher antioxidant potential due to greater light exposure. However, just two out of four studies found anthocyanin and flavonoid content in berry skin and polyphenol contents in wine to differ between organic and conventional management. Further investigations are necessary to understand possible interactions among management systems, trellis systems, and varieties.

Many studies that assessed wine quality and wine sensory characteristics among conventional, organic, and biodynamic viticulture are inconsistent in their findings. More research is needed on grape, juice, and wine compositional analysis to better understand how differences of sensory characteristics perceived by several panels in quantitative descriptive analyses can be supported with reasoning. Grapes, juices, and wines from replicated field trials with representative distribution of plots should be used for this to clearly relate the outcome to the different management practices.

Studies included in the meta-analysis were partially heterogenic and limited in number. Moreover, some locations, such as Europe, were overrepresented in the meta-analysis due to data availability and frequency of trials in this area comparing conventional and organic viticultural production. This study nonetheless did not assess any interactions between location of the trials and treatments, but locations differed in their growth level, yield level, and level of TSS in juice. This is why when calculating the ratio of organic to conventional growth rate, yield, and TSS, European results were overestimated.

Future research should concentrate on the optimization of organic and biodynamic viticultural practices in the different environments concerning macronutrient supply, disease incidence, yields, and cost structure. One focus of future research should be how to increase biodiversity in perennial cropping systems in comparison to habitats that are not used agriculturally. The impact of an increase in biodiversity on vine pests and diseases to determine the benefits of these ecosystem services is one other major issue for future research. On the other hand, possible interactions of the management systems with different varieties, trellis systems, soil types, rootstocks, and irrigation regimes should be detected to determine more effective viticultural management systems.

The comparison of biodynamic and organic viticulture showed similar characteristics. Two recent studies on organic and biodynamic viticulture observed significantly lower Ψ_{pd} in the biodynamic plots. One of the two studies observed lower stomatal conductance of the biodynamic plots. At the same time, a significant increase in leaf enzymatic activity of the biodynamic plots for Sangiovese was observed. One

hypothesis is that especially the horn silica preparation 501 made from quartz powder might upregulate plant defense mechanisms attributed to induced plant resistance. This again might have implications for berry composition under biodynamic management. These hypotheses need confirmation, especially because the only study that has assessed these phenomena did not have randomized field replicates. This is why these observations cannot be clearly attributed to the biodynamic treatment.

In the viticultural trials included in this study, the application of the biodynamic preparations was one characteristic of the biodynamic plots, but livestock, which is one essential component of a biodynamic farm, was not included. It is very difficult to include this in randomized scientific field trials. On-farm experiments with a scientific setup might be more suitable in order to depict biodynamic farming and to draw conclusions on this specific management system.

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Supplemental Data for:

Döring J, Collins C, Frisch M and Kauer R. 2019.

Organic and biodynamic viticulture affect biodiversity and properties of wine: A systematic quantitative review.

Am J Enol Vitic 70:221-242. doi: 10.5344/ajev.2019.18047.

Supplemental Table 1 Characteristics of the studies included in the meta-analyses and meta-regressions.

| Authors | Study years | Number of study years included | Location | Varieties |
|--------------------------|-------------|--------------------------------|---------------|--|
| Bagheri et al. (2015) | 2012-2013 | 2 | South Africa | Cabernet Sauvignon |
| Botelho et al. (2015) | 2011-2013 | 3 | Italy | Sangiovese |
| Collins, unpublished | 2009-2014 | 6 | Australia | Cabernet Sauvignon |
| Corvers (1994) | 1990-1992 | 3 | Germany | Riesling, Kerner |
| Danner (1985) | 1979-1983 | 5 | Austria | Grüner Veltliner |
| Döring et al. (2015) | 2010-2012 | 3 | Germany | Riesling |
| Döring unpublished | 2013-2016 | 4 | Germany | Riesling |
| Guzzon et al. (2015) | 2014 | 1 | Italy | Pinot blanc and Riesling |
| Hofmann (1991) | 1987-1989 | 3 | Germany | Riesling, Kerner |
| Kauer (1994) | 1989-1991 | 3 | Germany | Riesling, Müller-Thurgau |
| Linder et al. (2006) | 1998-2005 | 8 | Switzerland | Chasselas |
| Malusà et al. (2004) | 2000 | 1 | Italy | Grignolino |
| Meißner (2015) | 2006-2009 | 4 | Germany | Riesling |
| Picone et al. (2016) | 2009, 2011 | 2 | Italy | Sangiovese |
| Pool and Robinson (1995) | 1990-1994 | 5 | United States | Concord, Elvira, Seyval |
| Reeve et al. (2005) | 2000-2003 | 4 | United States | Merlot |
| Wheeler and Crisp (2011) | 1992-2006 | 15 | Australia | Cabernet Sauvignon, Merlot, Shiraz, Chardonnay |

Supplemental Table 2 Results of the balanced fixed factorial analysis of variance (ANOVA) and Tukey's test for the analysis of pruning weight, yield, and total soluble solids in juice comparing integrated or conventional and organic or biodynamic viticulture.

| Parameter | Treatment ^a | Int ^b (mean ± sd) | Org ^c (mean ± sd) | Continent | Interactions |
|--------------------------|------------------------|---------------------------------|---------------------------------|-----------|--------------|
| Pruning wt (t/ha) | *** | 3.22 ± 0.79 a ^d | 2.55 ± 0.71 b | *** | ns |
| Yield (t/ha) | ** | 11.94 ± 5.84 a | 9.92 ± 4.99 b | *** | ns |
| Juice sugar concn (Brix) | ns | 18.77 ± 3.59 - ^e | 18.91 ± 3.67 - ^e | *** | ns |

*** and ** indicate statistical significance ($p < 0.01$ and $p < 0.001$) of the main effects determined by ANOVA (ns = not significant).

^bInt = integrated or conventional treatment.

^cOrg = organic or biodynamic treatment.

^dDifferent letters indicate statistically significant differences ($p < 0.05$) for the fixed factor management system determined by Tukey's test.

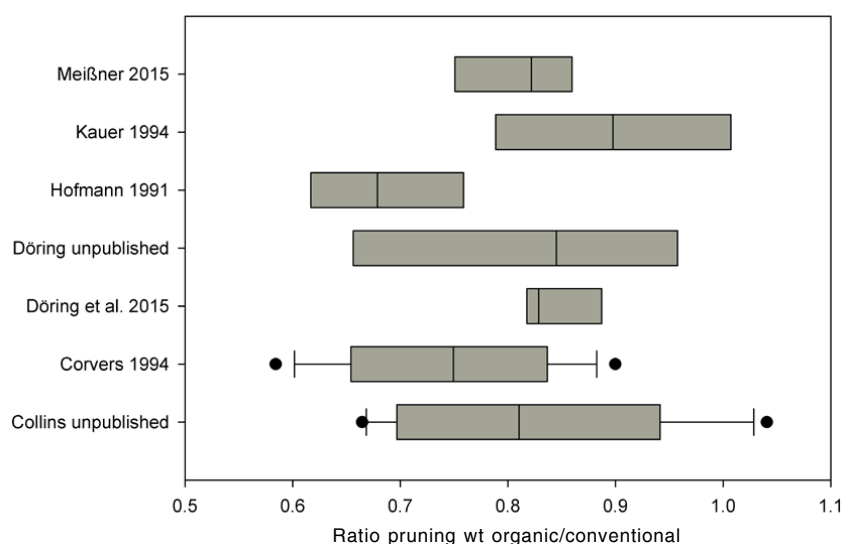
^eNo significant differences occurred between juice sugar concentration of juices from integrated and organic management.

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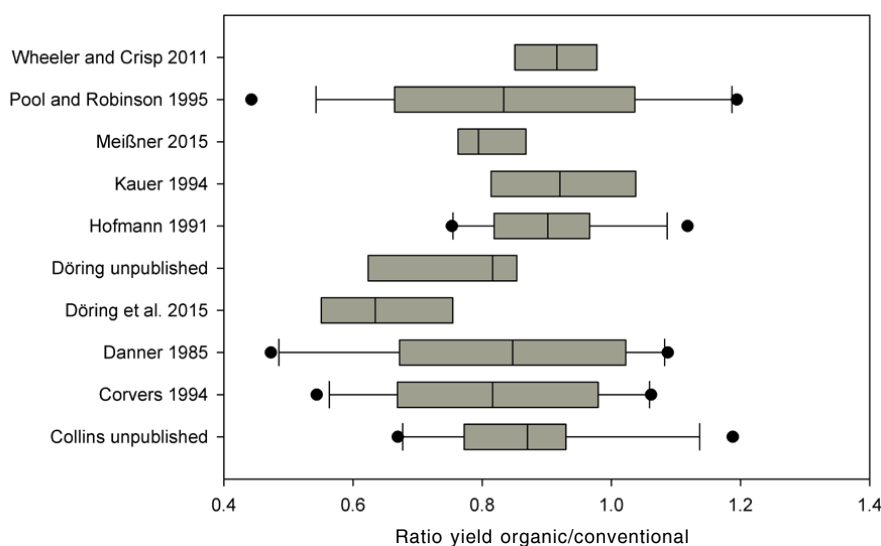
Döring J, Collins C, Frisch M and Kauer R. 2019.

Organic and biodynamic viticulture affect biodiversity and properties of vine and wine: A systematic quantitative review.

Am J Enol Vitic 70:221-242. doi: 10.5344/ajev.2019.18047.



Supplemental Figure 1 Ratio of pruning weight under organic compared to conventional management for every single study included in the meta-analysis and meta-regression. Bars express the distribution of the ratio of pruning weight for organic compared to conventional viticulture for every single study. Median Z with percentile Q0%, Q25%, Q75%, and Q100%, respectively (Köhler et al. 2007). Outliers are expressed as dots.



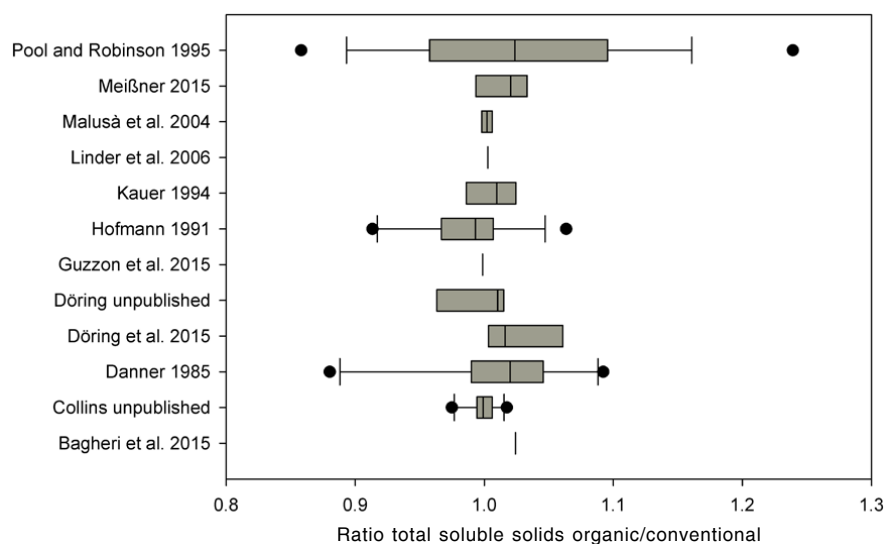
Supplemental Figure 2 Ratio of yield under organic compared to conventional management for every single study included in the meta-analysis and meta-regression. Bars express the distribution of the ratio of yield for organic compared to conventional viticulture for every single study. Median Z with percentile Q0%, Q25%, Q75%, and Q100%, respectively (Köhler et al. 2007). Outliers are expressed as dots.

Supplemental Data for:

Döring J, Collins C, Frisch M and Kauer R. 2019.

Organic and biodynamic viticulture affect biodiversity and properties of vine and wine: A systematic quantitative review.

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Supplemental Figure 3 Ratio of total soluble solids under organic compared to conventional management for every single study included in the meta-analysis and meta-regression. Bars express the distribution of the ratio of total soluble solids in juice for organic compared to conventional viticulture for every single study. Median Z with percentile Q0%, Q25%, Q75%, and Q100%, respectively (Köhler et al. 2007). Outliers are expressed as dots.

Chapter 6 General Discussion

Model for non-destructive Leaf Area Index measurements in the field needs further validation

A method for accurate, reliable and fast non-destructive Leaf Area Index (LAI) measurements in the field was established by gap fraction analysis in a VSP trained vineyard (*Vitis vinifera* L. cv. Riesling) using the portable Plant Canopy Analyzer (PCA, LAI-2200, LI-COR, Lincoln, NE, USA). Estimated Plant Area Index (PAI) measurements in the field were compared to directly measured LAI. All protocols tested need local calibration, since directly measured LAI differed from estimated PAI in all cases. The calibration equation for the measurement protocol chosen was established and the calibration allowed successful LAI measurements in experimental plots. The established method was used for assessing LAI under different management systems during the growing season 2013 (Fig.12).

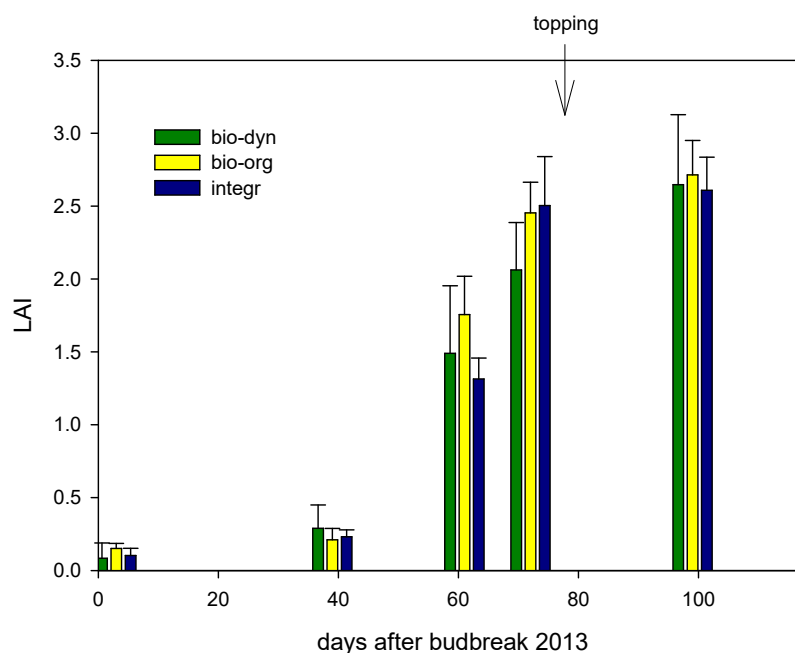


Fig.12: LAI under biodynamic, organic and integrated viticulture in 2013 (Döring unpublished).

Moreover, the method was applied to assess leaf area to fruit weight-ratio under biodynamic, organic and integrated viticulture in 2012 (Fig.13), as described in Chapter 4, and proved to be very useful for this purpose.

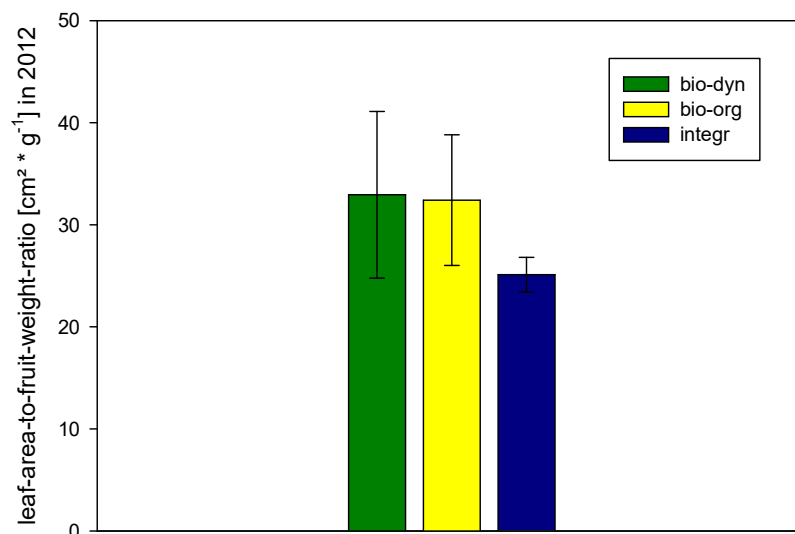


Fig.13: Leaf area to fruit weight-ratio of biodynamic, organic and integrated plots in 2012 [20].

No significant differences among treatments occurred, which might be one reason why fruit quality parameters did not show significant differences [20].

The established method showed to be a very useful tool for research purposes. It might also be suitable for practical approaches, since the instrument is easy to handle and the measurements are fast and simple. One drawback is that growers do not have access to the instrument and neither do they have experience with the measurement tool. It might be an interesting option for the use in chambers of agriculture or in large cooperatives. Since vine growth and vigor highly determine fruit quality and influence the health status of the grapes concerning *Botrytis* bunch rot and sour rot [46], the application of the protocol presented might be one possibility to assess target LAI values in vineyards of winegrowers delivering to cooperatives. For this purpose the protocol presented would need proper validation for other varieties, other training systems and other topographies (slopes). In 2013 the method was validated for two other training systems (split canopy Lyra; *Vitis vinifera* L. cv. Cabernet Sauvignon and minimal pruning in a VSP system; *Vitis vinifera* L. cv. Riesling) substantially differing in their canopy geometry (Fig. 14).

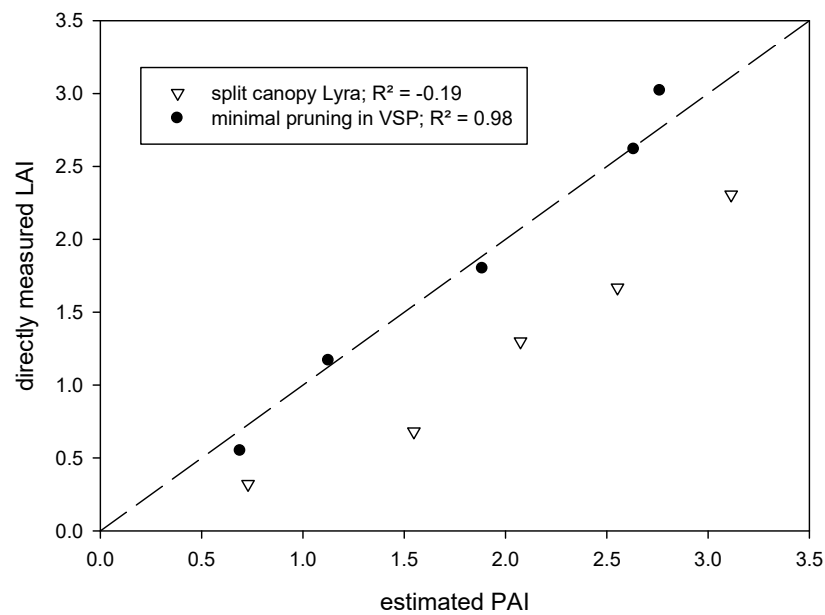


Fig. 14: Correlation between estimated PAI and directly measured LAI by destructive determination using the empirical calibration equation for protocol SFC (sensor facing canopy) for eight B-readings ($y = 1.1684x - 0.1809$) [21]; $n=5$ per training system; dashed line represents 1:1 relationship (Döring unpublished).

The correlation between estimated PAI and directly measured LAI (protocol SFC; eight B-readings) of minimal pruning in a VSP system using the empirical calibration equation established by Döring et al. [21] shows that the method could be validated successfully ($R^2 = 0.98$). This might be due to the fact that the canopy geometry of minimal pruning in a VSP system is similar to the VSP system of the vineyard the calibration was done in. The correlation between estimated PAI and directly measured LAI (protocol SFC; eight B-readings) of the split canopy Lyra using the same empirical calibration equation shows that, in contrast, the method could not be validated for this specific training system ($R^2 = -0.19$). PAI values of the split canopy are overestimated by the model used here. It is likely that this is due to the different canopy geometry of the split canopy Lyra in comparison to the vertical shoot positioning (VSP) system the calibration of the method was done in. By having a closer look at the evolution of estimated PAI throughout the transect it might be possible to explain why the protocol is not suitable for split canopies (Fig. 15).

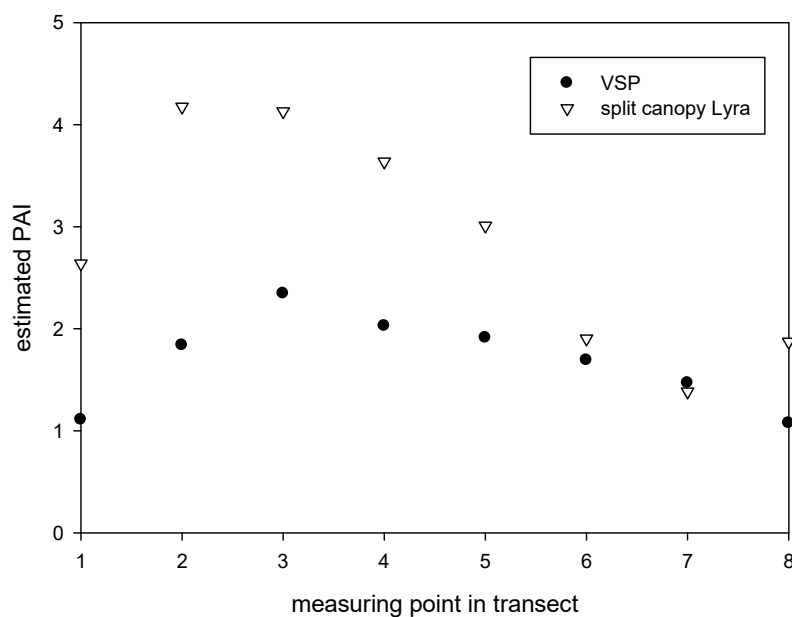


Fig. 15: Estimated PAI for every single measuring point in the transect for protocol SFC (sensor facing canopy) for eight B-readings (Döring unpublished).

The split canopy of the Lyra training system absorbs much more radiance in the first half of the transect compared to a VSP system. This is shown by much higher estimated PAI values of the split canopy. In split canopies a comparable leaf surface is distributed over a bigger canopy surface, clumping of leaves is less pronounced compared to a VSP system and light interception by the split canopy in relation to the leaf surface is much higher in relation to a VSP system. This might be the reason why the model overestimates PAI values of the split canopy compared to a VSP system.

The fact that in the split canopy Lyra the variety Cabernet Sauvignon was used is unlikely to account for the misfit of the model for LAI determination in this case, because the number and size of leaves between *Vitis vinifera* L. cv. Riesling in a VSP system also differs substantially from the one in a minimal pruning system in a VSP canopy. The minimal pruning system causes number of leaves to be higher and the average size of the leaves to be lower compared to a conventional VSP system even for the same variety (Döring unpublished). The leaf mean tilt angle is one other measure that can be determined with the Plant Canopy Analyzer (PCA, LAI-2200, LI-COR, Lincoln, NE, USA) and that might account for a possible misfit of the method and the respective calibration equation in the split canopy Lyra. It has neither been calibrated nor validated for grapevines in vineyards, but it does not show substantial differences among Cabernet Sauvignon in a split canopy, Riesling in a VSP system and Riesling in a minimal pruning system within a VSP canopy (Döring unpublished).

For a potential broader use of the model for LAI estimation it should be validated for other varieties and other training systems with a similar canopy geometry as the VSP

system. Training systems such as Cordon or Chablis used in Alsace and Champagne differing in canopy heights and distances sensor-canopy could provide suitable conditions for further validation.

Impact of the 'terroir' factors soil water-holding capacity and genotype on physiological performance of grapevines

As described in the introduction, soil and cultivar are two major terroir factors influencing plant performance and fruit quality. The contribution of grapevine genotype and soil water-holding capacity to the determination of plant physiological parameters was assessed to accurately describe the share of these two 'terroir' factors concerning plant performance under increasing drought. Two types of substrate were used in the experiment, a sand/clay mixture representing a WD soil and a clay substrate representing a WR soil. The WD soil was characterized by a much faster decrease of the matric potential and of the level of saturation when it dried compared to clay. Cabernet Sauvignon showed a more pronounced ABA-driven stomatal closure and a stronger downregulation of photosynthesis under increasing water stress confirming a near-isohydric behavior. The more anisohydric Syrah would be expected to generate a product of which characteristics strongly vary according to the substrate, its water-holding capacity and thus seasonal differences. This means that Cabernet Sauvignon would be expected to buffer vintage differences and preserve varietal characteristics independently from the water availability during the growing season.

Given the fact that the two cultivars Cabernet Sauvignon and Syrah show clear differences in the adaptation of stem water potential Ψ_{stem} to decreasing soil water potential Ψ_{soil} it seems necessary to split the data analysis and interpretation into one part with the reference value soil water potential and one part with the more plant-related reference value stem water potential in order to obtain accurate information about the contribution of the factors soil and cultivar to physiological performance and to gain more information about possible interactions which have not been considered in the current publication. ANOVA and Post-Hoc-Test (Tukey Test) were performed for stem water potential, stomatal conductance and PLC to assess the influence of the factors cultivar (grapevine genotype) and soil (soil water-holding capacity) on physiological performance and to assess their possible interactions for different water stress levels (Ψ_{soil}). For different stem water potential levels (Ψ_{stem}) ANOVA and Post-Hoc-Test (Tukey Test) were performed for the parameters stomatal conductance and PLC in order to assess the influence of the factors cultivar (grapevine genotype) and soil (soil water-holding capacity) and possible interactions.

Reference value soil water potential Ψ_{soil}

Under mild water stress levels the factor soil does not influence any of the physiological parameters assessed and the stem water potential is not influenced by the cultivar, neither. It does not show any significant changes at mild water stress levels (Table 2).

Table 2: Results of the balanced fixed factorial analysis of variance (ANOVA) and results of the Tukey's test for the fixed factors cultivar and soil and possible interactions under mild water stress ($\Psi_{\text{soil}} > -0.083$ MPa) (Döring unpublished).

| parameter | cultivar | Cabernet Sauvignon | Syrah | soil | water retaining | water draining | interaction cultivar:soil |
|--|----------|--------------------|---------|------|-----------------|----------------|---------------------------|
| stem water potential Ψ_{stem} [MPa] | n.s. | -0.972 | -0.764 | n.s. | -0.964 | -0.745 | n.s. |
| stomatal conductance g_s [mmol m ⁻² s ⁻¹] | ** | 36.1 b | 75.2 a | n.s. | 41.9 | 60.9 | n.s. |
| PLC [%] | * | 41.66 a | 33.38 b | n.s. | 39.99 | 38.81 | n.s. |

*, ** and *** indicate statistical significance ($p < 0.05$; $p < 0.01$ and $p < 0.001$) of the main effects determined by ANOVA (n.s. = not significant). Numbers indicate means per factor level and different letters indicate statistically significant differences ($p < 0.05$) per factor level determined by the Tukey's test.

Stomatal conductance and PLC are both influenced by the cultivar at these moderate water stress levels. The near-anisohydric Syrah also referred to as an 'optimist' shows significantly higher stomatal conductance and significantly lower PLC compared to Cabernet Sauvignon.

This result reveals that under mild water stress conditions no adaptation of stem water potential occurs. The significantly lower stomatal conductance and the significantly higher PLC of Cabernet Sauvignon seem to be stem-water potential independent, but characteristic for the near-isohydric variety. It could be deduced that under these mild water-stress conditions Syrah due to its higher stomatal conductance shows an optimal maintenance of the physiological traits and thus more growth or a potentially better nutrient supply of the grapes compared to Cabernet Sauvignon depending on the phenological stage of the vines. The water-holding capacity of the substrate does not play any role at these mild water stress levels meaning that these reactions are relatively soil-independent.

When water stress increases with decreasing soil water potential the cultivar significantly influences stem water potentials as well as PLC (Table 3).

Table 3: Results of the balanced fixed factorial analysis of variance (ANOVA) and results of the Tukey's test for the fixed factors cultivar and soil and possible interactions under intermediate water stress ($-0.083 \text{ MPa} > \Psi_{\text{soil}} > -0.212 \text{ MPa}$) (Döring unpublished).

| parameter | cultivar | Cabernet Sauvignon | Syrah | soil | water retaining | water draining | interaction cultivar:soil |
|---|----------|--------------------|----------|------|-----------------|----------------|---------------------------|
| stem water potential Ψ_{stem} [MPa] | * | -1.189 b | -0.875 a | * | -1.196 b | -0.867 a | n.s. |
| stomatal conductance g_s [$\text{mmol m}^{-2} \text{s}^{-1}$] | n.s. | 33.4 | 55.3 | * | 27.9 b | 60.8 a | n.s. |
| PLC [%] | * | 29.27 b | 38.32 a | * | 36.47 b | 38.15 a | n.s. |

*, ** and *** indicate statistical significance ($p < 0.05$; $p < 0.01$ and $p < 0.001$) of the main effects determined by ANOVA (n.s. = not significant). Numbers indicate means per factor level and different letters indicate statistically significant differences ($p < 0.05$) per factor level determined by the Tukey's test.

Stem water potentials of Cabernet Sauvignon are significantly lower compared to Syrah, but at the same time Cabernet Sauvignon shows lower PLC and thus less embolism formation compared to Syrah. This might be a hint that vessels of Cabernet Sauvignon are less prone to cavitation or that regulation of stomatal conductance in leaves of Cabernet Sauvignon is very effective under intermediate water stress levels and induces protection against embolism formation. Stomatal conductance under these intermediate water stress conditions is not significantly different, but still Cabernet Sauvignon shows lower average stomatal conductance compared to Syrah. PLC in Cabernet Sauvignon under intermediate water stress conditions is lower than under mild water stress conditions, which could be a further hint to its isohydric behavior preventing embolism formation under decreasing soil water potential. PLC in xylem vessels of Syrah, in contrast, is higher under intermediate water stress conditions indicating a more hydraulically-based decrease of stomatal conductance which is in accordance with a more anisohydric behavior in the presence of increasing water stress.

This result confirms that the two cultivars Cabernet Sauvignon and Syrah show clear differences in the adaptation of stem water potential to decreasing soil water potential. The reaction of Cabernet Sauvignon is much faster characterized by a faster decrease of stem water potential when soil water potential decreases during the experiment. On the other hand Cabernet Sauvignon shows a more pronounced decrease of stomatal conductance g_s when moderate water stress occurs. This might be related to its higher energy requirement to keep the sap flow under increasing stress conditions and is a characteristic behavior of near-isohydric cultivars. At the same time Cabernet Sauvignon seems to prevent excessive embolism formation by tightly regulating stem water potential and decreasing levels of stomatal conductance under intermediate water stress conditions. Under these conditions Cabernet Sauvignon might preserve soil moisture better compared to Syrah and might prevent excessive cavitation in xylem vessels to occur. It seems more adapted to increasing drought compared to Syrah. A

faster downregulation of growth in Cabernet Sauvignon might be one hypothetical consequence of this behavior implicating a potentially more 'pessimistic' interaction with available soil water. This behavior might be an advantage under increasing drought, since it ensures maintenance of physiological functions. It potentially implies a better nutrient supply of the ripening grapes after veraison if water stress occurs at these late phenological stages.

The soil type determines all three physiological parameters assessed here. Vines on WR soils show lower stem water potentials as well as lower stomatal conductance, together with slightly lower PLC. On WD soils plants still show higher stem water potentials and higher stomatal conductance, but slightly higher PLC. This might indicate the capacity of WR soils to trigger chemical signaling by submitting the roots to transient drought conditions, as already mentioned by [89].

Under severe water stress the factor cultivar influences stem water potentials and stomatal conductance, but does not have any significant influence on PLC and embolism formation any more (Table 4).

Table 4: Results of the balanced fixed factorial analysis of variance (ANOVA) and results of the Tukey's test for the fixed factors cultivar and soil and possible interactions under severe water stress ($\Psi_{\text{soil}} < -0.212$ MPa) (Döring unpublished).

| parameter | cultivar | Cabernet Sauvignon | Syrah | soil | water retaining | water draining | interaction cultivar:soil |
|--|----------|--------------------|----------|------|-----------------|----------------|---------------------------|
| stem water potential Ψ_{stem} [MPa] | ** | -1.78 b | -1.087 a | n.s. | -0.994 | -1.498 | n.s. |
| stomatal conductance g_s [mmol m ⁻² s ⁻¹] | * | 14.7 b | 35.2 a | n.s. | 19.5 | 22.3 | n.s. |
| PLC [%] | n.s. | 50.43 | 44.38 | * | 38.73 b | 51.28 a | n.s. |

*, ** and *** indicate statistical significance ($p < 0.05$; $p < 0.01$ and $p < 0.001$) of the main effects determined by ANOVA (n.s. = not significant). Numbers indicate means per factor level and different letters indicate statistically significant differences ($p < 0.05$) per factor level determined by the Tukey's test.

Cabernet Sauvignon shows significantly lower stem water potentials and significantly lower stomatal conductance compared to Syrah. This is a further hint to its near-isohydric behavior. Nevertheless under severe drought no difference in PLC and thus embolism formation between grapevine genotypes can be observed. Still lower stem water potentials leading to lower stomatal conductance in Cabernet Sauvignon might be favorable under severe drought and might delay irreversible cell plasmolysis irrespective of the soil water-holding capacity.

Under severe water stress the soil type solely influences PLC, but neither stem water potential nor stomatal conductance. Plants on WD soil show significantly higher PLC and thus significantly more embolism formation compared to WR soils. This means that the rupture of sap flow within xylem vessels is more likely to occur on WD soils independently from the grapevine cultivar.

Interactions between cultivar and soil did not occur neither under mild, nor under intermediate or under severe water stress. This means that both cultivars investigated showed their characteristic behavior independently from the water-holding capacity of the substrate. This is quite surprising and has not been understood before. Since Cabernet Sauvignon shows a tighter stomatal control under decreasing water availability and WR soils also seem to trigger this kind of tight stomatal control characterized by low stem water potentials, low stomatal conductance and a relative prevention of cavitation under intermediate water stress it could be hypothesized that in Cabernet Sauvignon on WR soils this behavior is even more pronounced. This does not seem to be the case. The more anisohydric Syrah on WR soils seems as prone for showing this more 'pessimistic' behavior as is Cabernet Sauvignon. This means that under increasing drought the grapevine genotype and the soil water-holding capacity both play a major role in determining physiological plant performance and thus fruit quality. In contrast, under mild water stress conditions when water availability is not a limiting factor, the cultivar and not the soil mainly influences physiological plant performance. Under severe water stress conditions both factors have a significant impact on physiological plant performance again. Since Cabernet Sauvignon on WR soils shows the lowest stomatal conductance and the lowest absolute PLC, this might be the set-up under which physiological functions could potentially be maintained longest under severe drought.

Considering the different characteristic traits of the two grapevine genotypes and the two types of substrate investigated it can be deduced that there are optimal set-ups of combinations of genotype and water-holding capacity, but interactions between genotype and substrate have not been observed. Every combination genotype-soil shows its characteristic traits described above. According to the prevailing environmental conditions concerning water availability optimal setups exist, which do not only depend on water availability and grapevine genotype. Other parameters such as climatic conditions (e.g. relative humidity, distribution of precipitation throughout the season etc.) and management restraints (e.g. slope, cover crops, nutrient supply etc.) might play a decisive role in selecting a specific genotype for a site with its specific soil water-holding capacity. For maximum plant growth a grapevine cultivar classified as near-anisohydric should be selected under mild or no water stress on both WR and WD soils, whereas on a WR soil frequently subjected to severe water stress a grapevine cultivar classified as near-isohydric should be chosen to ensure maintenance of physiological performance under drought. It cannot be deduced that near-isohydric grapevine genotypes buffer vintage effects or express substrate-specific traits to a lesser extend

compared to Syrah. Syrah might have more specific requirements concerning the environmental conditions under which it shows optimal growth. Cabernet Sauvignon, in contrast, might be more universally usable compared to Syrah due to its characteristic behavior under drought. Both cultivars express their characteristic physiological behavior according to the extent of water stress to which they are subjected with different specific implications for plant performance and fruit quality.

In general the number of plants included in the current study seems quite low. In total 16 grapevines were used in the experiment, meaning that per combination substrate-genotype four plants were used ($n=4$). More replicates per treatment combination would be necessary to confirm results and conclusions of the current study. At least three groups of plants per combination substrate-genotype should be used to support the hypotheses formulated above and means per group should be used for data analysis in order to spatially average measured values of each parameter in a greenhouse set-up which of course cannot be homogeneous.

Reference value stem water potential Ψ_{stem}

In the current study stem water potential was used as reference value for comparing stomatal conductance and PLC for the two different grapevine genotypes on the two different substrates used. For a better understanding of the physiological response of the plants to increasing water stress two groups of stem water potentials were considered: $\Psi_{\text{stem}} > -1$ MPa and $\Psi_{\text{stem}} < -1$ MPa. But it was not taken into consideration that the stem water potential itself is a measure that is cultivar and soil dependent when intermediate water stress occurs, as shown above. Cabernet Sauvignon shows significantly lower stem water potential under increasing drought compared to Syrah, and plants on WR soils show significantly lower stem water potential compared to WD soils. Under severe water stress levels stem water potential is solely dependent on the grapevine genotype, but not on the soil water-holding capacity.

One consequence of this fact is that when statistically analyzing different groups of stem water potential levels according to soil and cultivar, as done in Fig. 5 and Fig. 6 of the current publication, different numbers n of every combination cultivar-soil are available. This is why for understanding if there are possible interactions it is more suitable to assess fitted curves. Zufferey et al. [103] assessed stomatal conductance g_s and PLC in relation to stem water potential Ψ_{stem} in an irrigation trial on field-grown *Vitis vinifera* L. cv. Chasselas plants and showed that the correlation between stem water potential and stomatal conductance g_s as well as PLC follows sigmoidal patterns. As shown in Fig. 16, every combination cultivar/soil in the current study had its own characteristic correlation between stem water potential and stomatal conductance and stem water potential and PLC, respectively.

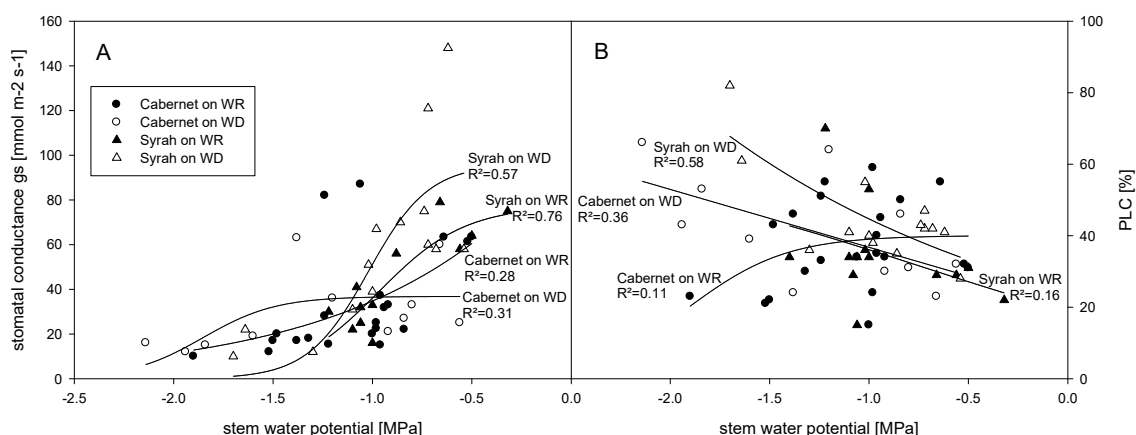


Fig. 16: Relation between stem water potential Ψ_{stem} and stomatal conductance g_s (A) and PLC (B), respectively, for the different combinations cultivar/soil of the current study. Curves represent sigmoidal growth curves (Döring unpublished).

This is not surprising since the stem water potential Ψ_{stem} itself is highly dependent on the grapevine genotype as well as on the soil water-holding capacity according to the level of soil water potential. It implies that when comparing stomatal conductance g_s and PLC for different combinations cultivar/soil data of different soil water potential levels are being compared.

In general stomatal conductance seems to be positively linked to stem water potential for all combinations cultivar/soil considered in the current trial, since coefficients of determination reach from $R^2=0.28$ to $R^2=0.76$ (Fig. 16). Stomatal conductance decreased with increasing xylem tension, thus decreasing stem water potential values, for all combinations cultivar/soil of the current trial. WD soils seem to maximize differences in stomatal conductance of the two cultivars in respect to WR soils, which seem to buffer plant reactions to water stress, maybe due to their characteristic trait of gradually releasing water to the plant.

The link between stem water potential and PLC is not as tight, since coefficients of determination R^2 are relatively low. Still PLC increases with high xylem tension, thus decreasing stem water potential levels, except for the combination Cabernet Sauvignon on WR soils. PLC for Cabernet Sauvignon on WR soils seems to be positively linked to stem water potential, although the correlation is rather weak ($R^2=0.11$), meaning that with increasing xylem tension PLC tendentially decreases instead of increasing. It was shown above that under increasing water stress PLC of Cabernet Sauvignon decreased and on WR soils PLC remained almost stable. The positive correlation of PLC with stem water potential for the combination Cabernet Sauvignon on WR soils might be the

expression of these phenomena observed above and might be linked to the isohydric behavior of Cabernet Sauvignon and to the capacity of WR soils to induce stomatal closure at intermediate water stress levels and to induce cavitation prevention under intermediate and severe water stress by lowering PLC, as shown above. Independently from the type of correlation between stem water potential and PLC the 50% PLC threshold is not reached on WR soils. For Syrah the 50% PLC threshold was reached when stem water potential Ψ_{stem} was about -1.1 MPa and for Cabernet Sauvignon it was reached at stem water potential Ψ_{stem} at about -1.9 MPa. Field-grown vines of the grapevine cultivar Chasselas reached the 50% PLC threshold at stem water potential Ψ_{stem} at -0.95 MPa [103], which is similar to a behavior of Syrah on WD soils of the current trial. This new approach to the data analysis reveals new perspectives on the diversity of the characteristic behavior of the two types of grapevine genotypes on the two substrates under water stress. Nonetheless all these observations need further confirmation and approval, since the number of plants included in the trial was low and thus influence of environmental factors cannot be excluded to a satisfactory extend.

Abscisic-acid related stomatal closure

The relation between ABA signals and stomatal conductance was also considered in the current study. Still the relation between stomatal conductance and ABA content in leaves should not be overrated in the current study, since mass spectrometry for distinct ABA identification was not coupled to the HPLC-DAD system used. Furthermore, the method by Materán et al. [57] was established for *Pinus radiata*, a coniferous evergreen tree, and has been adapted to *Vitis vinifera*. Moreover, the extraction of ABA from the leaf tissue requires many manual steps and can therefore be considered as relatively prone to potential errors. All this together with the low number of plants included in the trial underlines the necessity of further approval of the hypotheses formulated by applying a more resilient methodology coupled with a distinct identification of ABA.

Effects of organic and biodynamic viticulture – field trial in Geisenheim

Describing the effects of organic and biodynamic management on grapevine performance and fruit quality compared to the integrated management system was one major aim of this doctoral dissertation. Data of a field trial (*Vitis vinifera* L. cv. Riesling) comparing organic, biodynamic and integrated viticulture in Geisenheim, Rheingau, were collected over a three-year period (2010-2012) after conversion to characterize the effects of the respective management systems on growth, yield and fruit quality. During conversion vigor and yield decreased under organic and biodynamic

viticulture [63]. A decrease of growth and yield of grapevines under organic and biodynamic management compared to the integrated management was also observed in the current study after conversion was completed. Cluster weight as well as berry weight was reduced under organic and biodynamic viticulture. One parameter that might account for the changes observed is the physiological performance of the vines, which was enhanced under integrated management. On the other hand disease incidence and severity of downy mildew was increased under organic and biodynamic viticulture in two out of three years partially accounting for the yield reduction in the respective systems. Fruit quality (total soluble solids, total acidity, pH in healthy berries at harvest) and leaf area to fruit weight-ratio were not affected by the management system and use of biodynamic preparations had little effects on vine growth and yield.

The setup of the field trial corresponds to a complete block design. Factor levels of the main effect management system were replicated in four blocks. Each plot of one management system is composed of four rows with 32 vines each. Only the inner two rows of each plot were used for data collection, whereas the outer two rows of every plot were regarded as buffer rows.

A balanced fixed factorial analysis of variance was performed using the following model:

$$y = \mu + s_i + r_j + b_k + q_l + (sr)_{ij} + (sq)_{il} + e_{ijkl}$$

where μ is the mean, s_i ($i=1..3$) are the effects of the management system, r_j ($j=1,2$) are the effects of the rootstock, b_k ($k=1..4$) are the effects of the block, q_l ($l=1..3$) are the effects of the year, and e_{ijkl} is a random error term. The effects $(sr)_{ij}$ and $(sq)_{il}$ are interactions between the respective main effects. If a main effect or an interaction was significant ($p < 0.05$), a Post-Hoc-Test (Tukey test) was performed in order to compare factor levels. By having a closer look at the set-up of the trial there are several aspects which display clear drawbacks of the trial and reduce its explanatory power concerning the long-term effects of the respective management systems (Fig. 17).

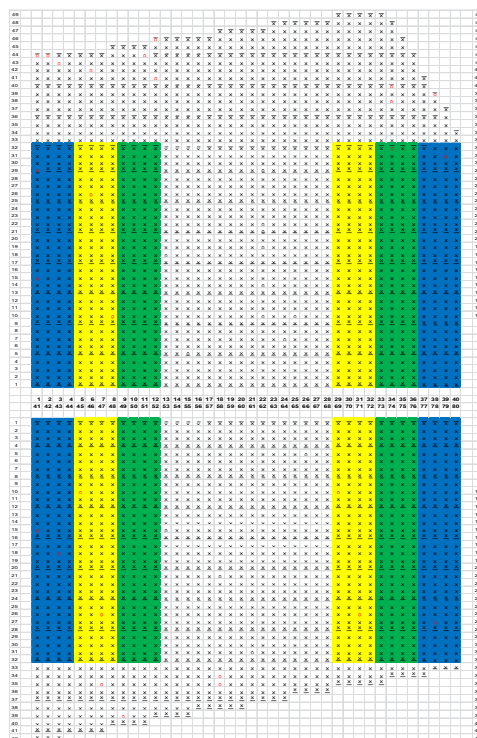


Fig. 17: Set-up of field trial in Geisenheim comparing integrated (blue), organic (yellow) and biodynamic (green) viticulture since 2006. x indicate single vines, o indicate missing vines and – indicate posts (Meißner unpublished).

First the total size of 0.8 ha of the plot divided into 20 sub-plots of approximately 300 m² of which 12 are used for the systems comparison of integrated, organic and biodynamic viticulture is quite limited. Even if the outer two rows of every sub-plot were not used for data collection, the risk of driftage of plant protection agents and mineral fertilizers is high since the width of one sub-plot is 8 m in average. This could have led to an influence of the respective adjacent sub-plots (neighboring effects) and could have potentially distorted results [87]. Yet in 2009 an analysis of residues of systemic plant protection agents on the clusters was carried out and no residues could be found in the organic and the biodynamic plots adjacent to the integrated plots [20,84].

Furthermore, it is a drawback that the sub-plots are not completely randomized. The fact that the principle of contingency was not followed when setting up the trial is an infringement of the prerequisite for an unbiased analysis of the data derived from the field trial [87]. The independence of the random effects is no longer given [87].

The field has been subdivided into four complete blocks. One major aim of setting up a complete block design is to record and to neutralize soil effects deriving from soil heterogeneity within the plot. The variability within the blocks must be smaller than the variability among the different blocks [87]. Prior to data collection in 2010 soil samples were taken and analyzed for differences among management systems and blocks,

respectively. Neither an influence of the block nor an influence of the management system was detected [20]. Still the distribution of the blocks should have been more carefully investigated before setting up the trial, for example by detecting possible gradients of soil quality parameters within the whole plot and by applying a nearest-neighbor analysis of yield data before starting the trial [87]. One possible consequence of this could have been a different distribution of the blocks along soil quality gradients and a closer positioning of the blocks for the systems comparison.

A compromise that has been made during the set-up of the trial was the linear distribution of the sub-plots leading to two respective field replicates with the same distribution of management systems. The advantage resulting from this specific distribution of the management systems was a reduction of transits by tractor. The path dividing the northern and the southern part of the plot is not as wide as to allow a tractor to turn. If the sub-plots of the respective management systems had been randomly distributed across the plot, one consequence would have been that the number of transits per row would have almost doubled. Taking into consideration that the trial had been planned and established as a long-term field trial from the beginning, it would have been negligent to accept a doubled number of tractor transits per row, which would have potentially enhanced soil compaction. On the other hand independence of random effects can just be guaranteed if random distribution of the sub-plots is fulfilled [87].

The biggest drawback concerning the set-up of the trial is the fact that the integrated management system lies on the outside of the plot in the eastern and the western part of the field. It is very likely that the outside position is linked to certain edge effects from the fields surrounding the whole plot. By purposeful choice of the integrated management system as the one lying on the outside of the plot on both sides these potential edge effects were automatically attributed to the respective management system. The assumption that the integrated management system could buffer potential effects coming from outside the plot leads to a bias in the field set-up [87].

An alternative set-up limiting the above-mentioned drawbacks and still reducing the transits of the tractor to a necessary minimum is presented in Fig. 18.

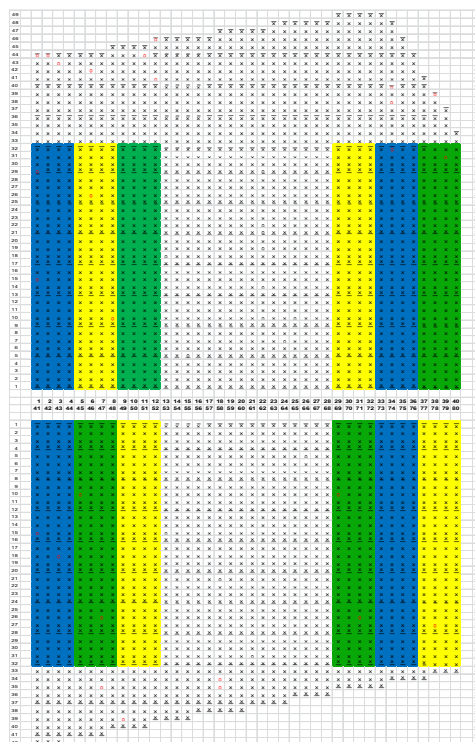


Fig. 18: Alternative set-up of field trial in Geisenheim comparing integrated (blue), organic (yellow) and biodynamic (green) viticulture. x indicate single vines, o indicate missing vines and – indicate posts (Döring unpublished).

Yet random distribution of the management systems within every block is not given, since the integrated treatment is still on the same position in the northern and the southern part of two respective field replicates. However, every management system lies on the outside of the plot in at least one field replicate assigning possible edge effects almost randomly to the management systems. The position of the organic and the biodynamic treatment could have easily been exchanged since the only difference in the management between these two systems is the manual spraying of the preparations and the type of compost applied once every three to four years. Within this set-up every block would have had a different distribution of the management systems, although the integrated system would still have been on the same position in two out of four field replicates, respectively. On the other hand it seems reasonable and important to limit soil compaction within the systems to a necessary amount in order to detect long-term soil effects of the respective viticultural management systems.

Another drawback of the field trial is the variation of multiple parameters per treatment. This is at the same time one of its biggest advantages, since existing management systems are being compared. On the other hand it was shown in Chapter 4 that drawing conclusions on reasons for observed changes is extremely difficult since multiple management parameters that might interact are potentially responsible for the

performance of one specific management system. For not only comparing, but advancing every single management system in terms of efficiency and fruit quality it is crucial to understand which management parameter within the system has major impact for example on physiological performance of the plants. One possibility to partially overcome this dilemma of the systems comparison trial would be to establish a fourth integrated variant applying mineral fertilizers and synthetic fungicides but using the cover crop mixture rich in legumes (Wolff-mixture) applied in the organic plots. The establishment of such a treatment would partially allow drawing conclusions on the contribution of the cover crop mixture to water availability in the soil, plant water potentials, physiological performance of the vines and growth on one hand and to fruit set and fruit quality on the other hand. One further reason for the establishment of the integrated treatment including the application of a Wolff-mixture is that cover crop mixtures rich in legumes are widely used in Germany not only among organic, but also among conventional winegrowers [66,101].

As mentioned in the conclusions section of the current article, further research on secondary metabolites in the berries originating from the different management systems would be crucial to understand whether the differences in growth observed cause any differences in the microclimate of the bunch zone within the canopy and thus induce changes for example in the flavonoid metabolism. The formation of these phenolic compounds related to radiation interception and translucency of the bunch zone might be affected by the different vigor within the management systems investigated.

The annual production of experimental wines per treatment over the complete duration of the trial since its beginning in 2006 has led to an interesting set of wines. This set of wines produced according to a standardized protocol should be used to understand whether the different management systems influence wine quality, which parameters are mainly affected and if there are changes that occur after a longer establishment of a certain treatment independently from vintage differences. At the same time this set of experimental wines should be used for targeted sensory evaluation aiming at answering the question whether differences in the sensory characteristics of the wines from the different management systems occur and if so whether these differences can potentially be linked to the analytical outcomes. This might be a suitable subject for a next PhD project at Hochschule Geisenheim University.

Global effects of organic and biodynamic viticulture

The necessity for such an investigation on wine quality and wine sensory characteristics under organic and biodynamic viticulture taking into consideration a big dataset of wines from a field trial obtained by a standardized protocol is also underlined by the

publication presented in Chapter 5 on the systematic quantitative review on effects of organic and biodynamic viticulture.

Clear regularities on soil nutrient cycling, biodiversity, growth, yield and total soluble solids could be established through this review. Soil nutrient cycling was enhanced under organic viticulture especially after conversion was completed. Cover crop mixtures used, compost application as well as the absence of herbicides might be factors that account for higher biological activity in organically and biodynamically managed soils, but the increase of soil nutrient cycling under organic and biodynamic management seems to take several years. In 17 out of 24 studies a clear increase in biodiversity under organic viticulture was observed on different trophic levels. Plant protection regime and cover crop mixtures seem to mainly determine higher biodiversity in organic and biodynamic viticulture. Biodiversity of less mobile taxa was more affected by the different management systems. Organic and biodynamic treatments showed 21 % lower growth and 18 % lower yield compared to conventional viticulture. A decrease in soil moisture content and physiological performance under organic and biodynamic viticulture is likely to be responsible for the lower growth and yield in the respective management systems and might be linked to the use of cover crop mixtures in organic and biodynamic viticulture. Juice total soluble solids concentration did not differ among the different management systems. It was also shown by the review that environmental and 'terroir' factors clearly influenced pruning weights, yields and total soluble solids within the different management systems investigated. On the other hand no interactions occurred. This implies that the management systems performed equally in the different environments and terroirs included in the review concerning pruning weight, yield and total soluble solids.

The review embedded the results obtained in the Geisenheim field trial in a global context underlining its exceptional position and its merits concerning the research on reasons for the observed changes in growth and yield on one hand. On the other hand the review contributed to a broader understanding of the management systems concerning nutrient cycling and biodiversity, two fields within which little research was done in the Geisenheim trial so far. Concerning wine quality and sensory characteristics in particular no consistent results could be provided by the review. One reason for this might be the difficult definition of wine quality, as mentioned above. Some studies report differences among management systems, but no overall consistent differences in berry composition, juice or wine quality among management systems could be observed. The big dataset of wines from the Geisenheim trial should be used to fill this gap of research. Furthermore, other investigations on sets of wines derived from field trials comparing the respective management systems should be conducted since the review underlined that environmental and 'terroir' factors influence growth, yield and total soluble solids in the management systems even though no interactions occurred. This means that results concerning organic and biodynamic viticulture obtained within a specific terroir and environment can be generalized. Still the characteristics of the

respective management systems might manifest themselves on different levels depending on the environmental and 'terroir' factors.

Performance of integrated, organic and biodynamic viticultural systems within different terroirs might be mediated by physiological performance and PLC

It was shown that under identical environmental conditions in the field trial in Geisenheim, Germany, physiological performance of integrated, organic and biodynamic viticulture differed significantly. In another field trial in Australia comparing conventional, organic and biodynamic viticulture under completely different environmental conditions it was observed that a decrease in soil moisture content in organic and biodynamic viticulture occurred in relation to conventional viticulture. This might be due to the use of cover crops in organic and biodynamic viticulture that consume more water and nutrients compared to the conventional or integrated variants, even though under Australian conditions cover crops die out at a certain point during the growing season. It can be hypothesized that physiological performance might mediate between soil moisture content and growth of the respective management systems. The characteristic behavior of different grapevine cultivars under certain soil water potentials described above might have to be extended to different management systems within certain environments. It should be further investigated whether physiological performance and embolism formation (PLC) are suitable parameters for describing the influence of different environmental conditions and terroirs on growth and yield within different management systems. This would be an important tool to understand and predict plant performance of the different management systems within terroirs worldwide that are characterized by a wide range of soil water potentials. This again would be of great importance to adapt and improve organic and biodynamic viticultural management systems as they are applied worldwide.

In the field trial in Geisenheim, Germany, physiological performance and embolism formation were measured during daily courses within the growing season 2012 (Fig. 19).

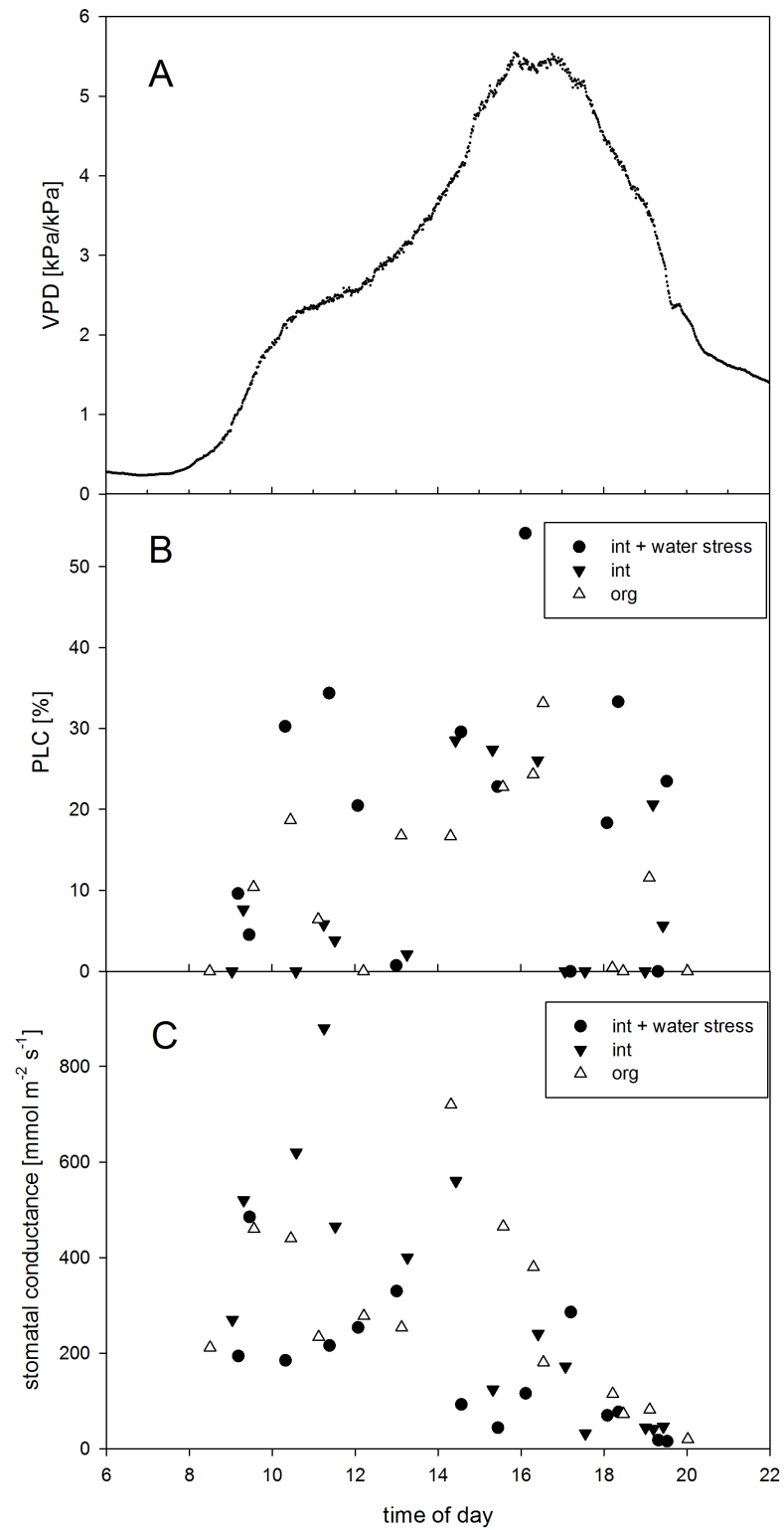


Fig. 19: Vapor pressure deficit (A), embolism formation (B) and physiological performance (C) under integrated (with and without water stress) and organic management in the field trial in Geisenheim, Germany, on 08/19/12 (*Vitis vinifera* L. cv. Riesling) (Döring unpublished).

The vapor pressure deficit VPD describes the evolution of vapor pressure to which the plants are subjected during the daily course. The evolution of embolism formation and repair (percent loss of conductivity PLC) and physiological performance (stomatal conductance g_s) are highly linked to the daily course of vapor pressure deficit. Comparing the data to data obtained by Zufferey *et al.* [103] for *Vitis vinifera* L. cv. Chasselas in Leytron, Switzerland in 2009 similar patterns of VPD and PLC can be observed. VPD on 08/19/12 in Geisenheim, Germany, reaches a maximum between 4 and 5 pm, whereas on 08/24/09 in Leytron, Switzerland, it reaches a maximum between 11 am and noon. Maximum VPD on 08/19/12 in Geisenheim is higher compared to maximum VPD on 08/24/09 in Leytron. Maximum values of embolism formation (PLC) are highly linked to VPD in both cases. In Geisenheim they occur between 2 and 5 pm, whereas in Leytron they occur between noon and 3 pm [103]. For non-irrigated, plastic covered field-grown Chasselas vines in Leytron maximum PLC is between 60 and 90 % and for irrigated field-grown Chasselas vines maximum PLC is between 10 and 20 % [103]. For non-irrigated, plastic covered field-grown Riesling vines (with water stress) maximum PLC is between 33 and 54 %, for non-irrigated, integrated field-grown Riesling vines (without water stress) maximum PLC is between 26 and 29 % and for non-irrigated, field-grown organic Riesling vines maximum PLC is between 22 and 33 %. The daily course of stomatal conductance g_s for field-grown *Vitis vinifera* L. cv. Riesling vines shows the characteristic pattern of maximum physiological activity before noon and after 2 pm interrupted by a midday depression linked to high temperatures on one hand and shading of the canopy within north-south oriented rows on the other hand [27]. Patterns of field-grown Riesling vines of the different treatments (integrated, integrated and plastic-covered with induced water stress and organic) seem to differ (Fig. 19). The non-irrigated, plastic covered vines (induced water stress) show substantially lower stomatal conductance and highest PLC, whereas the integrated vines without plastic cover show the highest stomatal conductance and the lowest PLC in comparison. The organic treatment shows intermediate stomatal conductance and intermediate PLC. It should be further investigated whether plants under the different management systems differ significantly in their susceptibility to cavitation and in their physiological performance during the daily course. If so, mediation through hormonal root-to-shoot signals such as ABA should be further examined within the different management systems under the same environmental conditions. As mentioned before, this would be a potential tool to predict plant performance under different management systems within terroirs worldwide.

In general it has to be underlined that in the current dissertation only a small portion of potential influencing factors on plant performance and fruit quality of grapevines were considered. Soil water-holding capacity as part of the natural physical terroir and different aspects of the human terroir such as grapevine genotype and management systems varying in soil cultivation, cover crop mixtures, under-vine management, pest management and fertilization strategy were considered and it was described to which

extend they influence plant performance and fruit quality of grapevines. Other important influencing factors of the natural physical terroir such as climate (temperature, rainfall, solar radiation), geology and geomorphology, topography, soil aspects such as soil mineral composition, soil color, soil biological activity, soil temperature and human factors in terroir such as pruning systems, trellis systems and canopy management were not considered here. Yet it has to be underlined that these factors not considered here influence plant performance and fruit quality to a high extend and might even play a role in determining physiological performance and PLC of grapevines under different management systems within various environments.

Research on biodynamic farming

In this context it should be expressed that doing research on biodynamic farming itself is a challenge, since this specific management system is partially based on holistic assumptions that are not in conformity with scientific principles, such as the mode of action and the effects of the biodynamic preparations or the influence of celestial bodies on plant growth and performance. It is controversially discussed whether a management system based on mechanisms of action that cannot entirely be explained by scientific principles can be scientifically evaluated by using conventional scientific methods. On the other hand the biodynamic farming system in viticulture is gaining more and more importance and consumers as well as producers show increasing interest in an objective evaluation of this farming system. This is why it has been included into the current investigations and publications on different management systems. I hope that by scientifically evaluating this management system and by applying its practices and integrating its principles as good as one can into a scientific field trial this contributes to increase the understanding and the comprehension of anthroposophers as well as of the defenders of conventional agriculture towards each other. Early statements from scientists on the scientific evaluation of the biodynamic preparations clearly show this conflict already and proof that investigations on biodynamic farming have often been guided by ideology more than by scientific aspects. Ewald Könemann, a pioneer of organic agriculture, as mentioned before, states in the journal *Bebauet die Erde*:246 in 1932: „Wenn man die große Zahl von Aufsätzen und Vorträgen gegen die Anthroposophie verfolgt, und die nicht minder große Zahl der hin und her laufenden Entgegnungen, muss man feststellen, dass hüben und drüben in den wenigsten Fällen Partner gegenüber stehen, die dem Stoff gewachsen sind: entweder weil auf Seiten der alten Schule die Kenntnisse der Geistes- und kosmischen Wissenschaft, ihr Wirken und ihre Geschichte fehlt, oder weil geldliche, berufliche, oder gar politische Interessen dahinterstehen, oder auf der gläubigen Seite der Glaube und der Hang zum Okkulten zu stark ist, die Kenntnis der Landwirtschaft und der Praxis zu gering, oder weil Ehre und Beruf davon abhängen, um nicht zu sagen – ebenfalls in gewissen Fällen der Geldbeutel.“ [96].

Conclusions

Within the current dissertation a protocol for fast and accurate LAI estimation by gap fraction analysis in the vineyard was successfully established. Yet a calibration equation is required to provide accurate LAI estimates. The protocol has to be further adapted to other trellis systems, trunk and vine heights, planting densities and vineyard management strategies. A validation of the protocol for two other trellis systems (minimal pruning in VSP and split canopy Lyra) showed that the protocol with the calibration equation provided gives reliable and accurate LAI estimates for minimal pruning in VSP systems, but not for the split canopy Lyra, since this specific trellis system shows differences in the canopy geometry. For a broader use of the method the protocol should be further validated for other trellis systems. The protocol was successfully used in the field trial in Geisenheim, Germany, comparing integrated, organic and biodynamic management for dynamic LAI estimation throughout the growing season as well as for the calculation of leaf area to fruit weight-ratio at harvest. These parameters are important indicators for plant performance and fruit quality.

In the second part of the current dissertation it was shown in a greenhouse trial that the two 'terroir' factors grapevine genotype and soil water-holding capacity highly determine plant physiological performance under increasing drought. By splitting up the original data analysis into one part with the reference value soil water potential and one part with the reference value stem water potential it was possible to gain new insights into the contribution of grapevine genotype and soil water-holding capacity to plant performance and physiological activity of the vines. Considering the different characteristic traits of the two grapevine genotypes and the two types of substrate in relation to soil water potential it can be deduced that there are optimal set-ups of combinations of genotype and water-holding capacity, but interactions between genotype and substrate have not been observed. It cannot be deduced that near-isohydric grapevine genotypes buffer vintage effects or express substrate-specific traits to a lesser extend compared to near-anisohydric cultivars, as was originally done in Chapter 3. Syrah might have more specific requirements concerning the environmental conditions under which it shows optimal growth. Cabernet Sauvignon, in contrast, might be more universally usable compared to Syrah due to its characteristic near-isohydric behavior under drought. By observing the characteristic behavior of the different combinations cultivar/soil under water stress in relation to stem water potential a new perspective on the diversity of physiological traits and their dependency upon environmental conditions was revealed. Nonetheless, as mentioned before, all these observations need further confirmation and approval, since the number of plants included in the trial was low and thus influence of environmental factors in the greenhouse could not be excluded to a satisfactory extend.

Further investigations on the consequences of the observed physiological behavior concerning plant growth and fruit quality should be undertaken, especially considering the two types of substrates used in this study and the two different grapevine genotypes (near-isohydric and near-anisohydric). A lot of research has been done on implications of vine water status for plant performance and fruit quality (please see Chapter 1), but the link between the characteristic physiological behavior of near-isohydric and near-anisohydric grapevine cultivars and implications on fruit quality under increasing drought on different soils has not been examined yet.

Results of the field trial in Geisenheim, Germany, comparing integrated (managed according to the *code of good practice* [6]), organic and biodynamic viticulture (managed according to Regulation (EC) No 834/2007 and Regulation (EC) No 889/2008 and according to ECOVIN- and Demeter-Standards, respectively) revealed the characteristic behavior of the vines under the respective management systems as well as reasons for the characteristic behavior. Through the detailed description of plant performance and fruit quality within the respective management systems the current work provides guidance for growers as well as for researchers. Especially the investigation of possible reasons for the observed changes within the single management systems can provide helpful guidance to develop more effective, optimized viticultural systems. Having a closer look at the trial set-up it reveals some weak points and could have been optimized before starting the trial in order to draw more distinct conclusions on the performance of vines under the respective management systems. On the other hand a fourth variant combining integrated vineyard management with the use of cover crop mixtures rich in legumes could have been a powerful tool to accurately detect to which extent the parameter cover crop determines plant performance and fruit quality of the organic management systems. In the near future the set of wines derived from the field trial since its establishment in 2006 should be used to adequately describe the quality and sensory effects of the respective management systems, a question to which growers, consumers and researchers search an answer.

The review on organic and biodynamic viticulture also revealed this gap of knowledge about the management systems. A lot of information on global effects of the management systems on soil, biodiversity, plant growth, yield, and fruit quality was provided. Organic and biodynamic viticulture affect soil, biodiversity, growth and yield of grapevines. Growth and yield of grapevines was significantly reduced under organic and biodynamic viticulture, but the level of total soluble solids in juice was not affected by the management system. Cover crop mixtures used, compost application as well as the absence of herbicides might be factors that account for higher biological activity in organically and biodynamically managed soils. Plant protection regime and cover crop mixtures mainly determine higher biodiversity in organic and biodynamic viticulture. A decrease in soil moisture content and physiological performance under organic and biodynamic viticulture is likely to be responsible for the lower growth and yield in the respective management systems. No clear results on the influence on wine quality and

sensory properties of the wines could be produced. It should be underlined that more detailed information on the influence of organic and biodynamic viticulture on wine quality and sensory properties of the wines under different 'terroir' conditions should be gained. The review also revealed the dependency of growth, yield and fruit quality under differing management systems upon environmental conditions and 'terroir' factors. This is why I propose to introduce reliable parameters that were shown to determine plant performance and thus fruit quality such as physiological activity and PLC that could eventually be used to predict the behavior of vines under organic and biodynamic viticulture under differing 'terroir' factors worldwide and to optimize its practices according to the environmental conditions.

Chapter 7 Summary

Plant performance and fruit quality of grapevines are influenced by a wide range of 'terroir' factors [44,102]. These factors, namely climate, soil as well as agricultural practices often called the 'human factor in terroir' all determine final wine quality to a high extend [40,92]. The share of influence of these three factors on growth, yield and fruit quality differs substantially between the different parameters [39,93]. Water relations play a major role within the natural environmental and within the 'terroir' factors, since the influence of a lot of climatic, soil-related as well as management factors is mediated through the water status in the soil [93,94]. Vine water status highly determines plant performance and fruit quality [11,25,67].

Agricultural management is an important tool to adapt agricultural systems to specific environmental conditions [72]. By comparing different management systems several parameters are usually varied together among treatments, such as soil management, fertilization strategy, pest and disease management, application of compost and crop rotations of annual cropping systems [87]. The most wide-spread viticultural management systems today are integrated, organic and biodynamic viticulture, even though organic and biodynamic viticulture are gaining more and more importance and attention among consumers and producers in the last decades [45,50].

The aim of this thesis was to establish a method for fast and accurate LAI measurements in the vineyard on one hand and to describe the influence of 'terroir' factors and vineyard management systems on plant performance and fruit quality of grapevines on the other hand by assessing vine water status, physiological activity, standard agronomical parameters as well as LAI.

A reliable, fast and accurate method to non-destructively and dynamically assess leaf area was established which can be applied in a small scale in the field. Different protocols for indirect estimation of leaf area index (LAI) by gap fraction analysis in VSP trained grapevines (*Vitis vinifera* L. cv. Riesling) were tested and correlated to direct leaf area measurements. LAI measurements were carried out using the portable Plant Canopy Analyzer (PCA, LAI-2200, LI-COR, Lincoln, NE, USA). One optimized protocol was chosen, the calibration equation was determined and the protocol was successfully used in the field trial in Geisenheim, Germany, comparing integrated, organic and biodynamic viticulture to assess LAI throughout the growing season as well as for determining leaf area to fruit weight-ratio at harvest. A validation of the protocol for two other trellis systems (minimal pruning in VSP and split canopy Lyra) showed that the protocol with the calibration equation provided gives reliable and accurate LAI estimates for minimal pruning in VSP systems, but not for the split canopy Lyra, since this specific trellis system shows differences in canopy geometry. For a broader use of the method the protocol should be further validated for other trellis systems.

Within the second approach of this doctoral dissertation the influence of the 'terroir' factors soil water-holding capacity and grapevine genotype on plant performance was assessed. For this purpose a greenhouse trial was set up comparing the physiological behavior of two grapevine genotypes (Cabernet Sauvignon and Syrah, near-isohydric and near-anisohydric) on two different soil substrates differing in their capacity to withhold water (water-draining and water-retaining). Stem-water potentials together with stomatal conductance and embolism formation (percent loss of conductivity PLC) were determined to describe physiological response mechanisms of the two different grapevine genotypes on two soil substrates. Under mild water stress conditions the cultivar and not the soil mainly influenced physiological plant performance. Under increasing drought the grapevine genotype and the soil water-holding capacity both played a major role in determining physiological plant performance. Cabernet Sauvignon showed a more pronounced stomatal closure and a stronger downregulation of photosynthesis under increasing water stress confirming a near-isohydric behavior compared to Syrah. Under intermediate water stress grapevines of both cultivars on WR soils showed a tighter stomatal control characterized by low stem water potentials, low stomatal conductance and a relative prevention of cavitation. Considering the different characteristic traits of the two grapevine genotypes and the two types of substrate in relation to soil water potential it can be deduced that there are optimal set-ups of combinations of genotype and water-holding capacity, but interactions between genotype and substrate did not occur. Syrah might have more specific requirements concerning the environmental conditions under which it shows optimal growth. Cabernet Sauvignon, in contrast, might be more universally usable compared to Syrah due to its characteristic near-isohydric behavior under drought. Yet all these observations need further confirmation and approval, since the number of plants included in the trial was low and thus influence of environmental factors in the greenhouse could not be excluded to a satisfactory extent.

The main objective of the third study included in the current doctoral dissertation was to determine growth, yield and fruit quality of grapevines under organic and biodynamic management in relation to integrated viticultural practices. Moreover, the mechanisms for the observed changes in growth, yield and fruit quality should be investigated by determining nutrient status, physiological performance of the plants and disease incidence on bunches in three consecutive growing seasons. A field trial (*Vitis vinifera* L. cv. Riesling) was set up at Hochschule Geisenheim University, Germany, in 2006. The integrated treatment was managed according to the *code of good practice* [6]. Organic and biodynamic plots were managed according to Regulation (EC) No 834/2007 and Regulation (EC) No 889/2008 and according to ECOVIN- and Demeter-Standards, respectively. The growth and yield of grapevines was significantly reduced under organic and biodynamic viticulture, but fruit quality was not affected by the management system. Physiological performance was significantly lower in the organic and the biodynamic systems, which may account for differences in growth and cluster

weight and might therefore induce lower yields of the respective treatments. Soil management and fertilization strategy could be responsible factors for these changes. Yields of the organic and the biodynamic treatments partially decreased due to higher disease incidence of downy mildew. The organic and the biodynamic plant protection strategies that exclude the use of synthetic fungicides are likely to induce higher disease incidence. Use of the biodynamic preparations within the biodynamic plots had little influence on vine growth and yield. Due to the investigation of important parameters that induce changes especially in growth and yield of grapevines under organic and biodynamic management the study can potentially provide guidance for defining more effective farming systems. Yet one major drawback of the trial is its set-up of non-randomized complete blocks which assigns possible edge effects to the integrated management system exclusively.

For a broader evaluation of the effects of organic and biodynamic viticulture compared to integrated or conventional viticulture a review of currently available literature was included into the doctoral dissertation. Effects of conventional, organic and biodynamic viticulture on soil properties, biodiversity, vine growth and yield, disease incidence, grape composition, sensory characteristics and wine quality were assessed and summarized. Only studies with representative field replicates or studies with a representative number of samples were included into the review. Yet studies were partially heterogenic and limited in number and publication bias cannot be excluded. Soil nutrient cycling was enhanced under organic viticulture especially after conversion was completed. 17 out of 24 studies observed a clear increase in biodiversity under organic viticulture on different trophic levels. Organic and biodynamic treatments showed 21 % lower growth and 18 % lower yield compared to conventional viticulture. The decrease of growth and yield under organic and biodynamic viticulture was not correlated to the growth or yield level under conventional viticulture. Juice total soluble solids concentration did not differ among the different management systems. No overall differences in berry composition, juice or wine quality among management systems could be observed. Cover crop mixtures used, compost application as well as the absence of herbicides might be factors that account for higher biological activity in organically and biodynamically managed soils. Plant protection regime and cover crop mixtures mainly determined higher biodiversity in organic and biodynamic viticulture. A decrease in soil moisture content and physiological performance under organic and biodynamic viticulture is likely to be responsible for the lower growth and yield in the respective management systems. Further research on wine quality and wine sensory characteristics under the respective viticultural management systems should be done since no clear conclusions could be drawn. In order to predict the behavior of vines under organic and biodynamic viticulture in different 'terroirs' worldwide parameters mediating between terroir and plant performance and thus fruit quality such as physiological activity and PLC could eventually be used. This would provide the

possibility to further adapt the practices of organic and biodynamic viticulture to environmental conditions worldwide.

Kapitel 8 Zusammenfassung

Das Pflanzenwachstum und die Fruchtqualität landwirtschaftlicher Nutzpflanzen wird von einer Vielzahl an sogenannten 'Terroir'-Faktoren beeinflusst [44,102]. Diese Faktoren, nämlich Klima, Boden und die Bewirtschaftung, die oft als 'menschlicher Terroir-Faktor' bezeichnet wird, bestimmen auch die Weinqualität maßgeblich [40,92]. Die 'Terroir'-Faktoren beeinflussen Wachstum, Ertrag und Fruchtqualität zu sehr unterschiedlichen Anteilen [39,93]. Unter den Umweltfaktoren und den 'Terroir'-Faktoren spielt der Wasserhaushalt eine große Rolle, da dieser eine Vermittlerrolle zwischen vielen Klima-, Boden- und Bewirtschaftungsfaktoren und dem Pflanzenwachstum einnimmt [93,94]. Der Wasserhaushalt der Rebe beeinflusst sowohl das Pflanzenwachstum als auch die Fruchtqualität maßgeblich [11,25,67].

Die Bewirtschaftung einer landwirtschaftlichen Fläche ist ein bedeutendes Instrument zur Anpassung landwirtschaftlicher Systeme an bestimmte Umweltfaktoren [72]. Beim Vergleich verschiedener landwirtschaftlicher Bewirtschaftungssysteme werden üblicherweise verschiedene Parameter zusammen variiert, wie z.B. Bodenpflege, Düngung, Pflanzenschutzstrategie, Kompostgabe und Fruchtfolge einjähriger Kulturen [87]. Die heute am meisten verbreiteten weinbaulichen Bewirtschaftungssysteme sind der integrierte, ökologische und biodynamische Anbau, wobei ökologischer und biodynamischer Weinbau in den letzten zwei Dekaden bei Konsumenten sowie bei Produzenten mehr und mehr an Bedeutung und Aufmerksamkeit gewinnen [45,50].

Das Ziel dieser Doktorarbeit war einerseits die Etablierung einer schnellen und zuverlässigen Methode der LAI-Messung im Weinberg und andererseits die Beschreibung des Einflusses von 'Terroir'-Faktoren und weinbaulicher Bewirtschaftung (ökologisch, biodynamisch) auf das Pflanzenwachstum und die Fruchtqualität der Rebe mittels Bestimmung des Wasserhaushalts der Reben, der physiologischen Aktivität, des LAI und weiterer pflanzenbaulicher Kenngrößen.

Eine robuste, schnelle und genaue Methode zur nicht-destruktiven und dynamischen Erfassung der Blattfläche in kleinen Weinbergspartellen wurde etabliert. Dazu wurden verschiedene Messprotokolle zur indirekten Schätzung des Blattflächenindex (LAI) mittels 'gap fraction analysis' in einem Weinberg mit Spaliererziehung (*Vitis vinifera* L. cv. Riesling) getestet und mit direkten, destruktiven Blattflächenmessungen korreliert. Die LAI-Messungen wurden mit dem tragbaren Plant Canopy Analyzer (PCA, LAI-2200, LI-COR, Lincoln, NE, USA) durchgeführt. Eines der Protokolle wurde ausgewählt, optimiert, kalibriert und das Protokoll wurde erfolgreich im Feldversuch in Geisenheim zum Vergleich des integrierten, ökologischen und biodynamischen Weinbaus eingesetzt, um den LAI während der Vegetationsperiode und das Blatt-Frucht-Verhältnis bei der Lese zu bestimmen. Die Validierung des Protokolls für zwei weitere Erziehungssysteme (Minimalschnitt im Spalier und Lyra-Erziehung) ergab, dass das Protokoll mit der vorhandenen Kalibrationsgleichung den LAI bei Minimalschnitt

im Spalier zuverlässig und genau schätzt, während bei der Lyra-Erziehung die Blattfläche mittels der vorhandenen Kalibrationsgleichung überschätzt wird. Dieses Erziehungssystem weist mit einer geteilten Laubwand eine andere Laubwandgeometrie auf. Für eine umfassendere Nutzung der Methode sollte das vorhandene Protokoll für weitere Erziehungssysteme validiert werden.

Im zweiten Teil der vorliegenden Arbeit wurde der Einfluss der 'Terroir'-Faktoren Wasserhaltefähigkeit des Bodens und Rebsorte auf das Pflanzenwachstum bestimmt. Dazu wurde die physiologische Aktivität zweier Rebsorten (Cabernet Sauvignon und Syrah, iso- bzw. anisohydrisch) auf zwei unterschiedlichen Bodensubstraten, die sich in ihrer Wasserhaltefähigkeit unterschieden, im Gewächshaus verglichen mittels Stammwasserpotenzial, stomatäre Leitfähigkeit und Emboliebildung im Xylem (percent loss of conductivity PLC). Bei mildem Wasserstress beeinflusste die Rebsorte und nicht das Bodensubstrat die physiologische Aktivität der Reben maßgeblich. Unter gesteigertem Wasserstress waren sowohl die Rebsorte als auch das Bodensubstrat ausschlaggebend für die physiologische Aktivität der Reben. Unter moderatem Wasserstress zeigte Cabernet Sauvignon im Vergleich zu Syrah eine ausgeprägte Stomatakontrolle, was zu einer Verringerung der Photosyntheseleistung führte. Dieses Verhalten ist typisch für isohydrische Pflanzen. Außerdem zeigten unter moderatem Wasserstress beide Rebsorten auf dem Bodensubstrat mit hoher Wasserhaltefähigkeit eine engere Kontrolle der Stomata. Das drückte sich durch niedrige Stammwasserpotenziale, niedrige stomatäre Leitfähigkeit und niedriger Embolieneigung im Xylem aus. Betrachtet man die unterschiedlichen Verhaltensweisen der zwei Rebsorten auf den beiden untersuchten Bodensubstraten je nach Bodenwasserpotenzial, kann man davon ausgehen, dass es optimale Kombinationen aus Rebsorte und Bodensubstrat für die einzelnen Bodenwasserpotenzialniveaus gibt. Jedoch traten keine Wechselwirkungen zwischen Rebsorte und Bodensubstrat auf. Syrah hat für eine optimale Versorgung und ein optimales Wachstum spezifischere Ansprüche an die Umweltbedingungen. Cabernet Sauvignon dagegen ist universeller einsetzbar aufgrund seines charakteristisch isohydrischen Verhaltens unter Wasserstress. All diese Erkenntnisse bedürfen jedoch einer wissenschaftlichen Bestätigung, da die Anzahl von Reben im Versuch gering war und ein Einfluss der Umweltfaktoren im Gewächshaus nicht ausgeschlossen werden kann.

Die Bestimmung von Wachstum, Ertrag und Traubenqualität der Reben unter ökologischer und biodynamischer Bewirtschaftung im Vergleich zur integrierten Wirtschaftsweise war das Hauptziel der dritten Publikation. Außerdem sollten die Ursachen der Unterschiede in Wachstum, Ertrag und Traubenqualität zwischen den Bewirtschaftungssystemen beschrieben werden. Dazu wurde der Nährstoffhaushalt der Reben, die physiologische Aktivität sowie die Befallshäufigkeit und -stärke der wichtigsten Pilzkrankheiten der Rebe in drei aufeinander folgenden Versuchsjahren betrachtet. An der Hochschule Geisenheim wurde dazu in 2006 ein Feldversuch (*Vitis vinifera* L. cv. Riesling) etabliert. Die integrierte Variante wurde nach guter fachlicher

Praxis [6] bewirtschaftet. Die ökologische und biodynamische Bewirtschaftung erfolgte nach VO (EC) No 834/2007 und VO (EC) No 889/2008 und ECOVIN- bzw. Demeter-Standards. Wachstum und Ertrag der Reben unter ökologischer und biodynamischer Bewirtschaftung waren signifikant reduziert. Die Traubenqualität jedoch war nicht vom Bewirtschaftungssystem beeinflusst. Die physiologische Aktivität der Reben unter ökologischer und biodynamischer Bewirtschaftung war signifikant niedriger, was einer der Gründe für das reduzierte Wachstum und das reduzierte Traubengewicht dieser Varianten sein könnte. Auch der geringere Ertrag unter ökologischer und biodynamischer Bewirtschaftung mag teilweise dadurch hervorgerufen sein. Gründe für die verringerte physiologische Aktivität könnten die Bodenpflege und die Düngung sein. Die Erträge der ökologischen und biodynamischen Varianten waren teilweise auch aufgrund von Befall mit *Peronospora* (*Plasmopara viticola*) reduziert. Der Pflanzenschutz im ökologischen und biodynamischen Weinbau, der ohne synthetische Fungizide arbeitet, ist hier wahrscheinlich der wichtigste Grund für den teilweise erhöhten Befall. Der Einsatz der biodynamischen Präparate innerhalb der biodynamischen Bewirtschaftung hatte wenig Einfluss auf Wachstum und Ertrag der Reben. Die Beschreibung der Ursachen für die Unterschiede in Wachstum und Ertrag zwischen integrierter Bewirtschaftung einerseits und ökologischer und biodynamischer Bewirtschaftung andererseits kann dazu beitragen, Handlungsempfehlungen für die einzelnen Bewirtschaftungsformen zu entwickeln und die Effektivität der jeweiligen Bewirtschaftungssysteme zu steuern. Ein großer Nachteil des Feldversuchs ist sein Aufbau als nicht randomisierte Anlage mit vollständigen Blöcken, der zur Folge hat, dass mögliche Randeffekte ausschließlich dem integrierten Bewirtschaftungssystem zugeschrieben werden.

Für eine umfassendere Bewertung der Effekte ökologischer und biodynamischer Bewirtschaftung im Weinbau wurde ein Review der derzeit vorhandenen Studien in die vorliegende Dissertation integriert. Effekte der konventionellen, ökologischen und biodynamischen Bewirtschaftung im Weinbau auf Bodenparameter, Biodiversität, Rebenwachstum, Ertrag, Schaderregerbefall, Traubenqualität, Weinqualität und sensorische Eigenschaften der Weine wurden bestimmt und zusammengefasst. Lediglich Studien mit repräsentativen Feldwiederholungen oder Studien mit einer repräsentativen Anzahl an Proben wurden in das Review einbezogen. Die Nährstoffumwandlung durch Bodenmikroorganismen unter ökologischer und biodynamischer Bewirtschaftung ist speziell nach Abschluss der Umstellung erhöht. In 17 von 24 Studien konnte eine signifikante Erhöhung der Biodiversität auf unterschiedlichen trophischen Niveaus unter ökologischer und biodynamischer Bewirtschaftung festgestellt werden. Ökologische und biodynamische Bewirtschaftung zeigten global 21 % weniger Wachstum und 18 % weniger Ertrag im Vergleich zu konventioneller Bewirtschaftung. Diese Reduzierung von Wachstum und Ertrag war nicht abhängig vom jeweiligen Wachstums- oder Ertragsniveau der entsprechenden konventionellen Variante. Die Zuckerkonzentration im Most unterschied sich zwischen

den Bewirtschaftungssystemen nicht. Es konnten keine generellen Unterschiede in Beereninhaltsstoffen, Most- oder Weinqualität der unterschiedlichen Bewirtschaftungssysteme festgestellt werden. Die Begrünungsmischungen, der Stallmistkomposteinsatz und das Fehlen von Herbiziden könnten Faktoren sein, die die höhere biologische Aktivität der Böden im ökologischen und biodynamischen Anbau bedingen. Der Pflanzenschutz und die Begrünungsmischungen waren maßgeblich für eine erhöhte Biodiversität unter ökologischer und biodynamischer Bewirtschaftung verantwortlich. Das geringere Wachstum und der geringere Ertrag unter ökologischer und biodynamischer Bewirtschaftung scheinen vor allem durch eine Reduzierung der Bodenfeuchte und der physiologischen Aktivität der Reben verursacht zu sein. Weitere Forschung zu Weinqualität und sensorischen Eigenschaften der Weine unter konventioneller, ökologischer und biodynamischer Bewirtschaftung ist vonnöten, da die Ergebnisse aus bisher vorliegender Literatur keine eindeutigen Schlüsse zulassen. Um Wachstums- und Ertragseigenschaften der Reben unter ökologischer und biodynamischer Bewirtschaftung unter unterschiedlichsten Umweltbedingungen vorhersagen zu können, erscheint es plausibel, Messgrößen heranzuziehen, die eine Vermittlerrolle zwischen 'Terroir' und Pflanzenwachstum bzw. Fruchtqualität einnehmen, wie z.B. physiologische Aktivität und Emboliebildung im Xylem. Dadurch bestünde die Möglichkeit, die Praktiken des ökologischen und biodynamischen Weinbaus weiter an die Umweltgegebenheiten der unterschiedlichsten 'Terroirs' weltweit anzupassen.

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