



ORIGINAL RESEARCH ARTICLE

# The impact of sustainable management regimes on amino acid profiles in grape juice, grape skin flavonoids and hydroxycinnamic acids

Johanna Döring<sup>1\*</sup>, Frank Will<sup>2</sup>, Otmar Löhnertz<sup>3</sup>, Birgit Krause<sup>3</sup> and Randolph Kauer<sup>1</sup>

<sup>1</sup> Hochschule Geisenheim University, Institute of General and Organic Viticulture, Von-Lade-Str. 1, D-65366 Geisenheim, Germany

<sup>2</sup> Hochschule Geisenheim University, Institute of Beverage Research, Von-Lade-Str. 1, D-65366 Geisenheim, Germany

<sup>3</sup> Hochschule Geisenheim University, Institute of Soil Science and Plant Nutrition, Von-Lade-Str. 1, D-65366 Geisenheim, Germany

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\*correspondence:  
johanna.doering@hs-gm.de

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## ABSTRACT

Organic and biodynamic management practices in viticulture have been sharply increasing worldwide for almost two decades and are seen as one possibility for environmentally friendly production. Consumers often presume that the quality of organically grown crops is different or higher compared to conventionally grown crops.

In the current study, wine grape quality under integrated, organic and biodynamic viticulture was assessed in a long-term field trial in Geisenheim, Germany, starting seven years after conversion. Treatments substantially differed in their amino acid concentration in juice, flavonol content and their composition of hydroxycinnamic acids. Organic and biodynamic treatments showed significantly higher concentrations of all amino acids in juice and a significantly higher concentration of flavonols in berry skins. Major drivers of these changes are reductions of yield and pruning weight under organic and biodynamic management on the one hand and increased light exposure of bunches in the respective treatments on the other hand.

Linking amino acid composition in juice, grape skin flavonoid and hydroxycinnamate concentration to canopy structure, vigour and yield enables growers to actively control and manipulate wine grape quality in the different management systems.

**KEYWORDS:** organic, biodynamic, flavanols, flavonols, hydroxycinnamic acids, wine grape quality

## INTRODUCTION

One of the biggest challenges of agriculture today is maintaining food safety and food quality while providing ecosystem services such as biodiversity conservation, pest and disease control, ensuring water quality and supply and climate regulation (Bünemann *et al.*, 2018). Organic farming was shown to promote biodiversity and greenhouse gas sequestration on the one hand (Bengtsson *et al.*, 2005; Hole *et al.*, 2005; Skinner *et al.*, 2019) and was shown to reduce inputs of nutrients and fossil energy on the other hand (Mäder *et al.*, 2002) and is, therefore, seen as one possibility of environmentally friendly production. Demand and production of organically grown crops have sharply increased in the last two decades (Willer *et al.*, 2021). Ultimately the EU has set the goal of at least 25 % organic farming until 2030 in their Green Deal's Farm to Fork strategy (European Commission, 2020). Organically managed viticulture surface has increased from 88,000 ha in 2004 to almost 468,000 ha in 2019 (Willer *et al.*, 2021). The share of organically managed viticulture surface worldwide (6.7 % by 2019) is much higher compared to the organically managed agricultural surface of annual crops (Willer *et al.*, 2021) but still has not reached 25 % yet.

Consumers expect organically grown crops to be free from chemical pesticides and mineral fertilisers and often presume that the quality of organically grown crops is different or higher compared to conventionally grown crops (von Meyer-Höfer *et al.*, 2015). While the absence of synthetic pesticides and mineral fertilisers is a prerequisite of organic farming worldwide (European Union, 2018), results concerning the food quality of organically grown crops are inconsistent (Mitchell *et al.*, 2007; Oliveira *et al.*, 2013; Reganold *et al.*, 2010; Reganold *et al.*, 2001; Spornberger *et al.*, 2007). Grape quality under conventional, organic and biodynamic viticulture has been the subject of investigation (Cravero, 2019; Döring *et al.*, 2019). Beyond parameters such as total soluble solids (TSS) and total acidity, amino acids are important factors for determining final wine quality because they serve as the main nitrogen sources for wine yeasts (Purificación Hernández-Orte *et al.*, 2002). Amounts and composition of amino acids are highly dependent on variety, rootstock, vintage and site and soil management (Choné *et al.*, 2001; Huang and Ough, 1989; Pérez-Álvarez *et al.*, 2015; Peyrot des Gachons *et al.*, 2005) and usually increase with increased nitrogen supply (Smit *et al.*, 2014). There are very few studies describing the influence of organic and biodynamic management on amounts of yeast available nitrogen (YAN) in organic and biodynamic viticulture. Meißner *et al.* (2019) and Collins *et al.* (2015) did not detect any differences in yeast available nitrogen content among conventional, organic and biodynamic treatments, whereas Döring *et al.* (2015) detected a significant increase of YAN in biodynamic viticulture after conversion. There is no report on the composition of amino acids in organic and biodynamic viticulture so far, but several studies dealt with the effects of fungicide application or the effect of light and temperature on amino acid composition in

grapes. Llorens *et al.* (2000) observed a negative effect of copper treatment on the amount of total nitrogen and free amino acids in roots and leaves of grapevines cultured in agar. Another study dealing with field-grown grapevines exposed to frequent copper treatments also reported a decrease in the content of free amino acids in mature grapes (Martins *et al.*, 2014). Oliva *et al.* (2011) detected a decreasing effect of various synthetic fungicides used in integrated viticulture on nitrogenous compounds in mature grapes under critical agricultural practice. Furthermore, amino acid concentration and composition have been shown to vary with the light interception of bunches in the canopy (Guan *et al.*, 2017).

Flavonoids protect plants from UV radiation due to their flavonoid ring structure, but many flavonoids and isoflavonoids also have phytoalexin activity acting against fungal or bacterial pathogens (Downey *et al.*, 2006). Due to their bitterness and astringency, flavan-3-ols and tannins may also deter herbivores (Downey *et al.*, 2006). Phenolics and polyphenolics have important antioxidant activity and can, therefore, induce health benefits (Buchner *et al.*, 2014; Rodrigues *et al.*, 2012; Shahidi and Ambigaipalan, 2015). Moreover, they are responsible for the sensory perception of wines, for colour, astringency and bitterness (Monagas *et al.*, 2005). In white wines, phenolics are usually perceived as negative influencing factors to the sensory profile, acting as browning precursors (Singleton *et al.*, 1975). Hydroxycinnamates are usually the most abundant class of phenolics in juice and thus in white wines. Hydroxycinnamic acids in berry skins can act as precursors of vinylphenols in the final wine and can be responsible for a depreciating phenolic sensory characteristic (Chatonnet *et al.*, 1993). Flavanols are considered to be precursors of tannin polymers. In grapes, flavanols are usually present as their corresponding glucosides, galactosides and glucuronides. Their amounts are highly dependent on light exposure (Adams, 2006). High levels of hydroxycinnamic acids and flavanols are usually considered to negatively affect final white wine quality (Choné *et al.*, 2006).

The formation of phenolic compounds in plants is highly linked to variety, temperature, light exposure, altitude, soil type, water and nutritional status and several biotic factors (Brandt *et al.*, 2019; Downey *et al.*, 2006; Friedel *et al.*, 2015; Price *et al.*, 1995). As these factors are closely related to each other and all together influence plant vigour and yield, the influence of every single parameter is hard to estimate (Downey *et al.*, 2006). Nitrogen levels have a fundamental influence on vine growth and vigour. Low and excessively high levels of nitrogen fertilisers lowered anthocyanin and flavonoid content in agricultural crops, including grape berries (Delgado *et al.*, 2004; Heimler *et al.*, 2017; Keller and Hrazdina, 1998; Kliewer, 1977). Vine vigour showed to positively correlate with flavan-3-ol monomers, whereas it is negatively correlated with berry skin proanthocyanins (Cortell *et al.*, 2005).

Total flavonol content in berry skins of Shiraz increased by applying early and late water stress. This might partially

be explained due to the decreasing effect of water deficit on berry size. Pulp weight is reduced by water deficit, whereas berry skin weight is only affected by early water deficit applied between flowering and veraison (Ojeda *et al.*, 2001). As a consequence of smaller berry size, the ratio of skin weight to total berry weight as well as the structure and development of the berry skin is changed, affecting anthocyanin and flavonoid concentration in the berry (Downey *et al.*, 2006; Roby and Matthews, 2004; Triolo *et al.*, 2019). In another study, flavonol concentration in the berry skin, as well as flavonol content per berry, was not affected by late water deficit in Cabernet-Sauvignon berry skins (Kennedy *et al.*, 2002). Total flavanol content in berry skins of Shiraz (per single berry) decreased with early-season water deficit, whereas late water deficit applied after veraison did not seem to influence flavanol content per berry in berry skins (Ojeda *et al.*, 2001). It is still controversially discussed whether, beyond this indirect berry size effect, flavonoid biosynthesis is directly affected by water deficit (Castellarin *et al.*, 2007; Downey *et al.*, 2006; Kennedy *et al.*, 2002). Post-veraison water deficit in Cabernet-Sauvignon showed that flavonols decreased (Kennedy *et al.*, 2002). Castellarin *et al.* (2007) showed limited effects of early and late water deficit on the flavonols in Cabernet-Sauvignon as well as on the expression of genes involved in their synthesis. Anthocyanin accumulation increased under water stress in the same study due to an earlier and greater expression of genes involved in the anthocyanin biosynthetic pathway (Castellarin *et al.*, 2007).

Light and temperature are two main determinants of berry composition. Early shading treatments showed a significant decrease in flavonoid content, but that revealed to be a temperature and humidity effect, as well, underlining the necessity to separate light and temperature effects (Buttrose *et al.*, 1971; Kliewer, 1977; Smart *et al.*, 1988). Wine grapes exposed to UV light showed higher flavonol glucosides than shaded fruit (Haselgrove *et al.*, 2000; Price *et al.*, 1995; Spayd *et al.*, 2002). Flavonol content in leaves and fruit of grapevine is shown to be highly light-dependent (Downey *et al.*, 2004b). Exposure of tissues led to an expression of the gene encoding flavonol synthase and flavonol glycosyltransferase (Downey *et al.*, 2004a; Friedel *et al.*, 2016). Flavanols and most hydroxycinnamic acids accumulate mainly before veraison, whereas flavonol synthesis occurs mainly post-veraison (Friedel *et al.*, 2015). This is why shading and leaf removal treatments after flowering did not substantially alter flavanol (except catechin) and hydroxycinnamic acid contents in Riesling berries, whereas flavonol content was highly increased due to leaf removal treatments (Friedel *et al.*, 2015).

Several studies detected an increase in anthocyanin and flavonoid content in berry skin, an increase in resveratrol content and enzyme polyphenol oxidase concentration in grapes, as well as an increase in polyphenol content, antioxidant potential and phenolic acid content in juice and wine from organic management (Artem *et al.*, 2021; Dani *et al.*, 2007; Granato *et al.*, 2015; Malusà *et al.*, 2004;

Miceli *et al.*, 2003; Otreba *et al.*, 2006; Tassoni *et al.*, 2014; Tintunen and Lehtonen, 2001; Toaldo *et al.*, 2015; Vrček *et al.*, 2011; Yıldırım *et al.*, 2004). Other studies did not find any evidence for a change in polyphenol or anthocyanin profiles of grapes and wines, their carotenoid and *trans*-resveratrol content, the content of *p*-coumaric acid or their antioxidant activity under organic viticulture (Bunea *et al.*, 2012; Collins *et al.*, 2015; Dutra *et al.*, 2018; Garaguso and Nardini, 2015; Heimler *et al.*, 2017; Lante *et al.*, 2004; Mulero *et al.*, 2009; Mulero *et al.*, 2010; Tassoni *et al.*, 2013). Two studies even observed a decrease in total polyphenol content and antioxidant activity or *p*-coumaric acid in wines originating from organic practices (Beni and Rossi, 2009; Yıldırım *et al.*, 2004), although very high amounts of manure were applied in the latter study. The adaptation of organic viticulture practices to local climatic and growing conditions might explain the inconsistency of these results. This is why it is even more important to correlate findings concerning wine grape quality to growth, vigour, yield, temperature, water stress and nutrient status of the plants to draw conclusions on which might be the main influencing factors.

There is a tight relation between the metabolic pathway of phenolic compounds and amino acids (Downey *et al.*, 2006). It can be deduced that both metabolic pathways compete for carbon skeletons. In fact, Friedel *et al.* (2015) observed a negative correlation between the accumulation of phenols and amino acids in different shading and leaf removal treatments in berries of Riesling.

The study aimed to investigate whether the different viticulture management systems integrated, organic and biodynamic differ in wine grape quality, more specifically in amino acid composition in juice, and flavonoids and hydroxycinnamic acids in berry skins. Furthermore, the authors wanted to unravel which variables, such as canopy structure, vigour, yield, canopy temperature or water stress, can account for changes in amino acid and phenolic composition in ripe grape berries from different management systems. For this purpose, the respective parameters were monitored during three consecutive seasons in a long-term field trial comparing integrated, organic and biodynamic viticulture management starting seven years after conversion. Linking amino acid composition in juice, grape skin flavonoid and hydroxycinnamate concentration to canopy structure, vigour and yield enables growers to actively control and manipulate wine grape quality in the respective management regimes.

## MATERIALS AND METHODS

### 1. Field trial

The field trial was located in Geisenheim (49° 59'; 7° 56'), is 0.8 hectares in size and was 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 vineyard had a spacing of 1.2 m within rows and 2 m between rows using a vertical shoot positioning system (VSP). Until 2005 the vineyard was managed according to the code of good practice (Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz, 2010). Conversion to organic and biodynamic viticulture started in 2006.

The experiment was set up as a complete block design. The three different management systems were replicated in four blocks. Each main plot for the factor management system was subdivided into two subplots, including the two different rootstocks, Börner and SO4, respectively. Each main 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 (Döring *et al.*, 2015).

A weather station located approximately 500 m from the trial site was used for climate data collection (Hochschule Geisenheim University, 2022).

## 2. Management

All plots were managed according to the code of good practice (Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz, 2010). In addition, organic and biodynamic plots were managed according to Regulation (EU) No 2018/848 (2018) of the European Parliament and of the Council of 30 May 2018 on organic production and labelling of organic products and repealing Council Regulation (EC) No 834/2007 and Regulation (EC) No 889/2008 and according to standards of the associations ECOVIN and Demeter, respectively.

Identical soil and vine management practices were applied to both organic and biodynamic plots, except that biodynamic preparations were only applied to the biodynamic plots. A species-rich cover crop mixture (Wolff-Mixture®, 50 kg ha<sup>-1</sup> sown on a width of 1.7 m within every row except the under-vine area) was used as a cover crop in both the organic and biodynamic plots (Döring *et al.*, 2015). The organic and the biodynamic treatments were supplied with nitrogen by breaking up the cover crop mixture (rich in legumes) of every second row shortly before full bloom. In the integrated plots, a sward mixture (ProGreen® WB 120 Mulchmischung II) was established as a cover crop in the inter-row space. The sward mixture in every second row of the integrated plots was ploughed shortly before bloom, together with the cover crop of the organic and the biodynamic plots. The integrated plots are amended with mineral fertilisers (25 kg N ha<sup>-1</sup> on 07/05/12 and 25 kg N ha<sup>-1</sup> on 06/16/14) to compensate for the higher nitrogen mineralisation in the organic and the biodynamic plots, which occurs due to the ploughing of the cover crop rich in legumes. Mineralised nitrogen content in topsoil was measured in all three growing seasons according to Schaller (2000) to assess the nitrogen demand of the respective treatments. Results from 2012 are shown exemplarily (Supplemental Figure 1).

In the organic and the biodynamic treatments, weeds were controlled mechanically in the under-vine area. Herbicides were used in the integrated treatment to control weeds under the vines.

Systemic fungicides were applied in the integrated plots to control powdery and downy mildew (*Erysiphe necator* and *Plasmopara viticola*). Magnesium nitrate fertiliser (0.25 %) was applied on 08/02/12 and 08/14/12; 07/24/13 and 08/05/13; iron chelate (13 %) was applied on 06/11/14. In addition, botryticides were applied twice a year.

To control powdery and downy mildew in the organic and the biodynamic plots, copper, sulphur and plant strengtheners (Mycosin VIN®, sodium bicarbonate, sodium silicate) were used. In all treatments, RAK® 1+2 M (500 dispensers ha<sup>-1</sup>; 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.

All three treatments received compost during the period of conversion (nitrogen equivalents) on 08/24/06 (50 kg N ha<sup>-1</sup>) and 10/24/07 (25 kg N ha<sup>-1</sup>). Farmyard manure was used for the organic and biodynamic plots (12.5 % N in fresh weight) and green waste compost for the integrated plots (21 % N in fresh weight). In addition, biodynamic compost preparations 502–507 were only applied to the biodynamic compost. No compost was applied to the plots during the current study period (2012–2014).

Each of the biodynamic field spray preparations (horn manure and horn silica) was applied three times a year. Horn manure was applied twice in spring and once after harvest and horn silica was applied at grapevine phenological stages shortly before full-bloom, at veraison and shortly before harvest. About 100 g ha<sup>-1</sup> of the cow pat pit preparation (Masson and Masson, 2013) was applied once a year in the growing season after tillage in years where no compost was applied to the biodynamic plots (Table 1).

## 3. Determination of yield, LAI, pruning weight, $\Psi_{pd}$

Crop yield was determined at harvest on 10/10/12, 10/15/13 and 10/02/14, respectively, on all vines per plot, leaving out the buffer rows. Leaf area index (LAI) was determined several times per season after full development of the canopy using the Plant Canopy Analyzer (PCA, LAI-2200, LI-COR, Lincoln, NE, USA) following the method described by Döring *et al.* (2014). Pruning weights of the two central rows per plot were determined gravimetrically in all three consecutive growing seasons. Pre-dawn water potentials ( $\Psi_{pd}$ ) were assessed on 06/19/12, 07/26/12, 08/24/13, 06/18/14 and 07/17/14 on mature, undamaged and non-senescent leaves using a pressure chamber (Scholander *et al.*, 1965) (Soilmoisture Corp., Santa Barbara, CA, USA) according to Turner (1988). Before the measurements, leaves were wrapped in polyethylene bags and detached from the primary shoots with a single cut.

**TABLE 1.** Management of integrated, organic and biodynamic plots in the current study.

	integrated	organic	biodynamic
Cover crops	Grass mixture	Wolff mixture	Wolff mixture
Under-vine management	Herbicides	mechanical	mechanical
Fertilisation	Mineral fertilisers and compost	Ploughing of cover crops and compost	Ploughing of cover crops and compost with biodynamic preparations
Plant protection	Synthetic fungicides, mating disruption (grape berry moth)	Copper, sulphur, plant strengtheners, mating disruption (grape berry moth)	Copper, sulphur, plant strengtheners, mating disruption (grape berry moth)
Biodynamic preparations	-	-	Horn manure, horn silica, compost preparations

#### 4. Determination of wine grape quality

Representative maturity sampling (100 berries per row on each date) took place on 10/10/2012, 10/08/2013 and 09/25/2014. The two canopy sides of the two central rows per plot were equally sampled. The mean single berry weight of the samples was determined gravimetrically. Berries were pressed at 1 bar (Longarone 85, QS System GmbH, Norderstedt, Germany) for two minutes the day after sampling. The obtained juice was analysed for total soluble solids (°Brix) by refractometry and for total acidity and pH by Fourier-transform infrared spectroscopy (FTIR) (FT2 Winescan, FOSS, Hillerød, Denmark) using an in-house calibration.

#### 5. Amino acid determination in grape juice

Grape juice samples of the respective treatments were extracted in lithium citrate buffer (2.2n, Sykam, Eresing, Germany) and subsequently filtered (syringe filter, nylon, 0.45 µm, MS Scientific, Berlin, Germany) for analysing unbounded amino acids. Chromatographic separation over two hours was performed on an amino acid analyser S433 (Sykam, Eresing, Germany) using a 4.6 × 150 mm LCA K 07/Li cation-exchange column (Sykam, Eresing, Germany). For quantification, automatic post-column ninhydrin derivatisation was applied before primary and secondary amino acids were detected photometrically at 570 and 440 nm, respectively, according to Krause and Löhnertz (2017). The evaluation took place with the software clarity amino (data apex, Prague, Czech Republic). Precision parameters were proved by constant participation in the worldwide scheme by ERNDIM (European Research Network for evaluation and improvement of screening, Diagnosis and treatment of Inherited disorders of Metabolism) through method comparison and certification of the determined physiological amino acid results.

#### 6. Phenolic composition in berry skins

For HPLC analysis of phenolic compounds, frozen berries were peeled in an oxygen-free CO<sub>2</sub> chamber. Berry skins were then freeze-dried and phenolic compounds were extracted from the freeze-dried skin powder in acidified acetonitrile under SO<sub>2</sub> protection, followed by vacuum distillation. After that, extracts were analysed by ACCELA HPLC/DAD system coupled to an LXQ mass spectrometer (ThermoFisher, Dreieich, Germany) according to Friedel *et al.* (2015).

#### 7. Determination of canopy architecture in the bunch zone

Canopy architecture in the bunch zone was determined by Point quadrat analysis (Smart and Robinson, 1991) with eight replicates per treatment of 50 insertions each shortly before harvest in 2012, 2013 and 2014, respectively. Spacing between insertions was 20 cm. The number of layers, leaf layers, internal leaves, gaps and clusters, as well as the number of exposed and shaded clusters were extracted from these data.

#### 8. Statistical analysis

Statistical analyses were carried out using the R software and the R Studio graphical user interface (R Core Team, 2018). Analyses of variance were calculated based on mixed linear models with management, rootstock and their interactions as fixed and block and year as random factors. In the case of LAI, analysis of variance was calculated based on a mixed linear model with management as fixed and block, year and date as random factors. In the case of  $\Psi_{pd}$ , analysis of variance was calculated based on a mixed linear model with management, rootstock and their interactions as fixed and block and date as random factors. Parameters that were significantly affected by the management system were subjected to the least significant difference in the post-hoc test.

Principal component analysis (PCA) was conducted using the R software and the R Studio graphical user interface. Data were autoscaled. PCA was calculated using packages `factoMineR`, `factoextra` and `ggplot2` (Kassambara, 2017).

For estimation of the effect of vintage and treatment on selected parameters of grape skin composition and amino acids in juice, a multiple linear regression analysis (function `lm`) was used.

## RESULTS

Long term annual rainfall for the site is 543 mm. Total rainfall in the three seasons 2012–2014 was 531 mm, 558 mm and 624 mm, respectively. Growing season rainfall was 330 mm, 397 mm and 446 mm for the seasons 2012–2014, respectively. Yields in the integrated treatment were 8039 kg ha<sup>-1</sup> on average from 2012 to 2014, whereas yields in the organic and the biodynamic treatments were, on average, 5159 and 5153 kg ha<sup>-1</sup>, respectively (Table 2). This corresponds

to a yield reduction in organic and biodynamic viticulture of 35.8 and 35.9 %, respectively. Pruning weights in the current study were rather high and differed significantly among treatments, with the integrated treatment showing significantly higher pruning weights compared to organic and biodynamic plots (Table 2). Leaf area in the growing season also differed among treatments. The integrated plots showed an average LAI of 1.43 and differed significantly from the biodynamic plots, which showed an average LAI of 1.27 (Table 2). Pre-dawn water potentials  $\Psi_{pd}$  ranged from  $-0.02$  to  $-0.56$  MPa between 2012 and 2014, indicating weak to moderate water stress (Spring and Zufferey, 2011). Pre-dawn water potentials  $\Psi_{pd}$  were slightly lower in the biodynamic treatment, but management systems did not differ significantly from each other (Table 2). Total soluble solids in juice at harvest reached 18 °Brix on average and did not differ among treatments throughout the period from 2012 to 2014. In contrast, treatments differed in the titratable acidity and pH in juice. Integrated plots showed significantly lower titratable acidity and, correspondingly, a significantly higher pH in juice compared to organic and biodynamic management regimes (Table 2). Berry weights at harvest ranged from 1.51 to 1.58 g berry<sup>-1</sup> on average. The organic and the biodynamic plots showed significant reductions in the berry weight compared to the integrated management practice (Table 2). The fresh weight of the berry skins, in contrast, did not differ among treatments (Table 2).

Concerning canopy structure in the bunch zone, treatments only differed in the number of layers and the number of clusters, but not in the number of leaf layers or internal leaves (Table 3). The organic and the biodynamic treatment showed 81.5 and 81 % of the number of clusters compared to the integrated treatment. The integrated treatment showed significantly more layers as well as more clusters compared to the organic and the biodynamic plots. This implies that

treatments did not differ in the number of leaves in the bunch zone but exclusively in the number of clusters in the bunch zone. This effect is even more evident when the number of exposed and shaded clusters per treatment is considered (Table 3). Treatments only differed in the number of shaded clusters, with the integrated treatment showing significantly more shaded clusters than organic and biodynamic treatments. Management regimes did not differ in the number of exposed clusters, but the share of exposed clusters was 28 % in the integrated treatment, 36 % in the organic treatment and 38 % in the biodynamic treatment.

Vintages highly differed in their concentrations of total amino acids in juice (Table 4), with 2013 showing the highest and 2014 showing the lowest concentrations of total amino acids in juice (Table S1). Organic and biodynamic treatments showed significantly higher concentrations of all amino acids compared to the integrated management regime (Table 4). The amount of total amino acids differed significantly among treatments. Organic and biodynamic treatments showed 1596.3 and 1582.4 mg L<sup>-1</sup> of total amino acids, whereas, in the integrated treatment, 982.2 mg L<sup>-1</sup> of total amino acids were detected on average (Table 4 and Table S1). This corresponds to a total amino acid-N of 282.5 and 278.6 mg L<sup>-1</sup> in the organic and the biodynamic treatment, respectively, and 163.8 mg L<sup>-1</sup> in the integrated treatment (Table 4). All three management systems thus showed sufficient N-supply for yeast nutrition to ensure successful fermentation (Dukes and Butzke, 1998). Arginine was the most abundant amino acid in all three treatments under investigation (int 210.5; org 393.9; bd 380.7 mg L<sup>-1</sup>), followed by proline (int 200.5; org 306.4; bd 301.3 mg L<sup>-1</sup>) and glutamine (int 119.6; org 232.9; bd 232.9 mg L<sup>-1</sup>) (Table 4). The share of proline, the only amino acid which is controversially discussed to be not yeast available (Smit *et al.*, 2014), was 14.9 % for integrated management compared to

**TABLE 2.** Effect of management system, rootstock, year, and interaction of management system and rootstock on yield, pruning weight, LAI, berry weight and juice quality from 2012 to 2014. In case of LAI, influencing factors are management, year and date.

	management	int	org	bd	rootstock	year	management:rootstock
Yield [kg ha <sup>-1</sup> ]	*	8039 a	5159 b	5153 b		*	
Pruning weight [dt ha <sup>-1</sup> ]	*	42.6 a	37.5 b	36.7 b	*	*	
Total soluble solids [°Brix]		17.95	18.13	18.23		*	
Titratable acidity [g L <sup>-1</sup> ]	*	12.32 b	12.81 a	12.88 a	*	*	
pH	*	2.86 b	2.89 a	2.89 a		*	
Berry weight [g]	*	1.58 a	1.51 b	1.51 b	*	*	
FW berry skin [g]		0.112	0.109	0.123		*	
	management	int	org	bd	year	date	
LAI [m <sup>2</sup> m <sup>-2</sup> ]	*	1.43 a	1.35 ab	1.27 b			
	management	int	org	bd	rootstock	date	management:rootstock
$\Psi_{pd}$ [MPa]		-0.191	-0.192	-0.198		*	

Analyses of variance were calculated based on linear mixed models. Significant factor effects (with  $\alpha = 0.05$ ) are marked with a \*. Average values are given per management system and different letters indicate statistically significant differences among factor levels in case of a significant management effect.

**TABLE 3.** Effect of management system, rootstock, year and interaction of management system and rootstock on canopy structure in the bunch zone from 2012 to 2014.

	management	int	org	bd	rootstock	year	management:rootstock
Layers	*	2.59 a	2.39 b	2.38 b		*	
Leaf layers		2.22	2.09	2.08		*	
Internal leaves		0.73	0.65	0.62		*	
Gaps		0.11	0.1	0.11		*	
Clusters	*	0.36 a	0.3 b	0.29 b		*	
Exposed clusters		0.1	0.1	0.11		*	
Shaded clusters	*	0.26 a	0.19 b	0.18 b		*	

Analyses of variance were calculated based on linear mixed models. Significant factor effects (with  $\alpha = 0.05$ ) are marked with a \*. Average values are given per management system and different letters indicate statistically significant differences among factor levels in case of a significant management effect.

**TABLE 4.** Effect of management system, rootstock, year and interaction of management system and rootstock on amino acids in juice at harvest [ $\text{mg L}^{-1}$ ].

	management	int	org	bd	rootstock	year	management:rootstock
Aspartate							
aspartic acid	*	37.4 c	42.3 a	39.7 b		*	
asparagine	*	4 b	8.8 a	8.9 a		*	
methionine	*	3.6 b	5.9 a	6.3 a		*	
threonine	*	41.2 b	62 a	62.5 a		*	
lysine	*	1.2 b	2 a	2 a		*	
isoleucine	*	16.5 b	24.6 a	26 a		*	
3-phosphoglycerate							
serine	*	42.6 b	71.7 a	71 a		*	
glycine	*	1.5 b	2.3 a	2.3 a		*	
Pyruvate							
alanine	*	49.7 b	78.3 a	79.7 a		*	
valine	*	19.2 b	28.1 a	28.9 a		*	
leucine	*	20.5 b	31.1 a	33 a		*	
Shikimate							
tyrosine	*	5.7 b	9 a	9 a		*	
phenylalanine	*	20.6 b	31.3 a	33.1 a		*	
tryptophan	*	8.1 b	14.6 a	15.4 a	*	*	
$\alpha$ -Ketoglutarate							
glutamic acid	*	56.4 b	78.7 a	77.5 a		*	
glutamine	*	119.6 b	232.9 a	232.9 a		*	
arginine	*	210.5 b	393.9 a	380.7 a		*	
proline	*	200.5 b	306.4 a	301.3 a		*	
histidine	*	10.6 b	18.3 a	18.5 a		*	
$\gamma$ -aminobutyric acid	*	49.1 b	68.3 a	70.3 a		*	
ornithine	*	0.97 b	1.63 a	1.59 a		*	
Total amino acids	*	982.2 b	1596.3 a	1582.4 a		*	
Total amino-N	*	163.8 b	282.5 a	278.6 a		*	
other							
ammonium	*	58.3 b	71.9 a	70.5 a	*	*	

Analyses of variance were calculated based on linear mixed models. Significant factor effects (with  $\alpha = 0.05$ ) are marked with a \*. Average values are given per management system and different letters indicate statistically significant differences among factor levels in case of a significant management effect.



13.2 % for organic and biodynamic treatments, respectively. Treatments differed clearly in the ratio of ammonium-N to total amino acid-N. The integrated treatment showed a ratio of ammonium N to amino acid-N of 27.7 %, whereas the organic and the biodynamic plots showed a ratio of 19.8 and 19.7 %, respectively. When yield levels of the single management systems are considered together with the amount of amino acid-N per hectare, the integrated treatment shows a lower amount of amino acid-N per hectare (840.2 g ha<sup>-1</sup>) compared to the organic (948.6 g ha<sup>-1</sup>) and biodynamic (934.5 g ha<sup>-1</sup>) plots.

Berry skins of the integrated treatment showed the highest concentrations of total hydroxycinnamic acids, whereas berry skins of the biodynamic treatment showed the highest concentrations of total flavonols. Treatments did not differ significantly in their concentration nor their composition of flavanols in berry skins (Table 5). Concentrations of flavanols were comparable to those of Brandt *et al.* (2019)

and Friedel *et al.* (2015). Treatments did not differ in their concentrations of hydroxycinnamic acids, but the composition of the latter differed significantly among treatments (Table 5). The concentrations of hydroxycinnamic acids again were comparable to those found by Friedel *et al.* (2015) and Brandt *et al.* (2019). The integrated treatment showed significantly higher concentrations of coumaroylglucose, pCGT and coutaric acid, whereas the organic and the biodynamic treatment showed significantly higher concentrations of glutathionylcaftaric acid and coutaroylglucose. Furthermore, the biodynamic treatment showed significantly higher concentrations of caffeic acid compared to organic and integrated plots. Concerning flavonols, which are known to be highly affected by light exposure, the organic and the biodynamic treatment showed significantly higher concentrations (org 959.3 µg g<sup>-1</sup> FW berry skin; bd 1071.6 µg g<sup>-1</sup> FW berry skin) compared to integrated plots (813 µg g<sup>-1</sup> FW berry skin) (Table 5). Concentrations of flavonols corresponded to control treatments of

**TABLE 5.** Effect of management system, rootstock, year and interaction of management system and rootstock on flavanols, flavonols, and hydroxycinnamic acids (µg g<sup>-1</sup> berry skin fresh weight).

	management	int	org	bd	rootstock	year	management:rootstock
Flavanols							
procyanidin B1		60.1	56.5	51		*	
catechin		38.8	42.5	39.8		*	
Procyanidin B2		72.4	77	66.3		*	
Epicatechin		19.1	21.7	18		*	
Total flavanols		190.4	197.6	175		*	
Hydroxycinnamic acids							
Cumaroylglucose	*	19.1 a	16.4 b	14.9 b		*	
Caftaric acid		524.7	510.9	502.1			
Glutathionylcaftaric acid	*	2.5 c	3.1 b	3.8 a		*	
p-CGT	*	13.5 a	12.3 b	11.2 b		*	
Coutaric acid	*	184 a	168.3 ab	159.6 b	*	*	
Caffeic acid	*	8.6 b	8.7 b	13.3 a		*	
Fertaric acid		13.8	15.1	12.8		*	
Coumaric acid		5	6.8	5		*	
Coutaroylglucose	*	47.2 b	62.3 a	62.3 a		*	
Total hydroxycinnamic acids		818.4	803.8	785.1		*	
Flavonols							
Que-3-rutinoside		68.3	76.9	81.3		*	
Que-3-galactoside		149.9	158.3	169.5	*	*	
Que-3-glucoside	*	286.8 b	371.1 a	405.7 a		*	
Que-3-glucuronide	*	136.8 b	168 ab	204.4 a		*	
Que-3-xyloside		33.6	38.6	41	*	*	
Que-3-arabinoside		89.9	87.4	97.9	*	*	
Que-3-rhamnoside	*	47.8 b	59.1 ab	71.8 a		*	
total flavonols	*	813 b	959.3 a	1071.6 a		*	

Analyses of variance were calculated based on linear mixed models. Significant factor effects (with  $\alpha = 0.05$ ) are marked with a \*. Average values are given per management system and different letters indicate statistically significant differences among factor levels in case of a significant management effect. P-CGT = p-coumaroylglucosyltartrate, Que = Quercetin.



Brandt *et al.* (2019) and Friedel *et al.* (2015). The integrated treatment showed significantly lower concentrations of Que-3-glucoside, Que-3-glucuronide and Que-3-rhamnoside. Management systems did not differ in the other flavonols assessed (Table 5). Since fresh weights of the berry skins at harvest did not differ among treatments (Table 2), it can be deduced that total amounts of flavanols and hydroxycinnamic acids did not differ. The three consecutive vintages considered differed in their total amounts of flavanols, hydroxycinnamic acids and flavonols, with 2012 showing the lowest concentrations of total flavanols, 2013 showing the lowest concentrations of total hydroxycinnamic acids and flavonols and 2014 showing the highest concentrations of both total hydroxycinnamic acids and flavonols (Table S1).

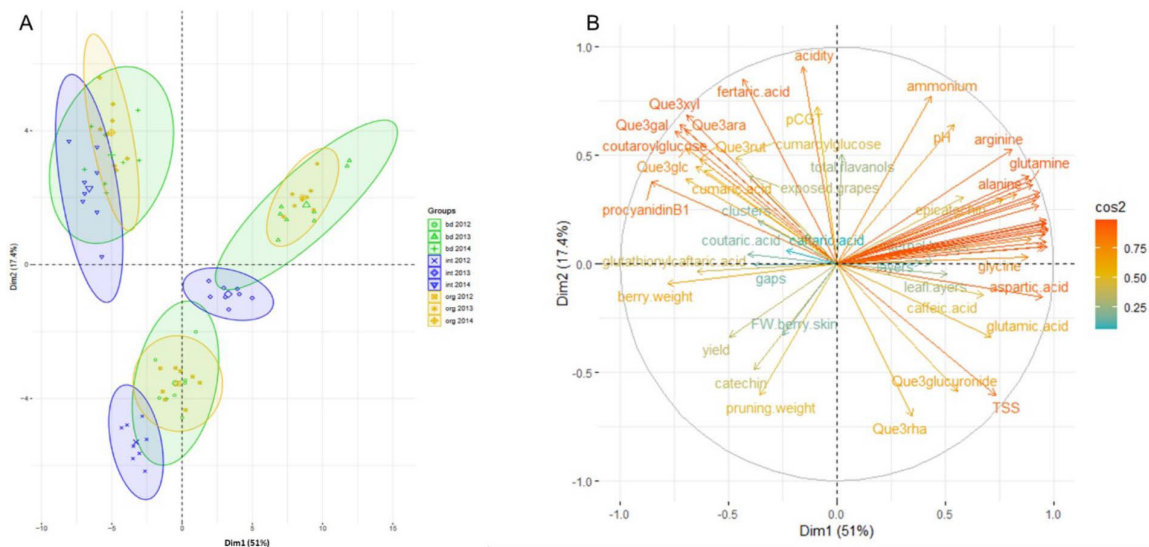
A PCA model was constructed with two latent variables explaining 68.4 % of x-block variation (Figure 1). Treatments were separated to a large extent by PC1 and to a minor extent by PC2 (Figure 1A). PC1 and PC2 both discriminated among vintages (Figure 1A). Treatments were mainly separated by the amount of total amino acids as well as by all amino acid concentrations, which were more abundant in organic and biodynamic plots (Figure 1B and Supplemental Figure S2). Major drivers of the separation of PC2 were total acidity, tartaric acid and ammonium (Figure 1B and Supplemental Figure S2). Total flavanols, caftaric acid, pCGT and fresh weight of berry skins had the smallest influence on PC1 and thus on the separations of the different management systems (Supplemental Figure S2). Yield and pruning weight were both negatively correlated to amino acid concentrations (Figure 1B).

## DISCUSSION

One essential difference between integrated management, on the one hand, and organic and biodynamic management, on the other hand, are the differences in yield, pruning weight and leaf area (LAI) (Döring *et al.*, 2015; Meißner *et al.*,

2019). A reduction in yield and vigour under organic and biodynamic viticulture compared to integrated management practices were already observed in several other studies (Döring *et al.*, 2019). Drivers for the reduction of yield, pruning weight and LAI in org and bd treatments seem to be a reduction in physiological performance (assimilation rate A, transpiration rate E, stomatal conductance  $g_s$ ), which did not always go along with lower water potentials and a lower Mg content in leaves at veraison (Döring *et al.*, 2015). These different levels of yield and pruning weight seem to be the main drivers for the differences in amino acid concentrations in juice and flavonoid composition in berry skins reported in the current study. Differences in mineralised nitrogen content in the soil cannot account for the differences in vigour and yield as the org and bd plots showed levels of NO<sub>3</sub>-N similar or higher compared to the int plots (Figure S1), despite the mineral fertilisation in the int plots in 2012 and 2014.

Amino acid composition blatantly differed among treatments. Organic and biodynamic treatments showed a significantly higher concentration of all amino acids and thus of total amino acids in juice. This implies that yeast nutrition during fermentation is favoured and the risk of stuck fermentation is much lower for organic and biodynamic juices compared to integrated juice, although all treatments showed sufficient N supply for successful fermentation (Dukes and Butzke, 1998). Still, a decrease in amino acid concentration in juice might affect the success of alcoholic fermentation (Bely *et al.*, 1990) and may also have implications on wine aroma and other beneficial effects such as protein synthesis (P. Hernández-Orte *et al.*, 2005). When amounts of total amino acid-N are considered together with yield levels, the integrated treatment shows a lower amount of amino acid-N per hectare. This means that yield levels alone cannot fully account for the differences in amino acid concentrations observed among treatments. This could potentially be a hint that the organic and the biodynamic systems are more



**FIGURE 1.** Scores plot (A) and loadings plot (B) of the principle component analysis (PCA) conducted on measurements from 2012 to 2014.

efficient in their nitrogen use compared to the integrated treatment. Both organic and biodynamic treatments did not receive any nutrient supply between 2012 and 2014, whereas, in the integrated treatment, 25 kg N ha<sup>-1</sup> as mineral N fertiliser was added both in 2012 and 2014. Organic and biodynamic plots were exclusively amended by ploughing the cover crop mixture, including legumes. This underlines that with adequate cover crop management in organic and biodynamic viticulture, N supply can be ensured under cool climatic conditions. On the other hand, synthetic pesticides might have a side effect on amino acid concentrations in grape juices. Especially fungicides that contain fenhexamid were reported to decrease amino acid concentrations in grapes (Oliva *et al.*, 2011). Teldor containing fenhexamid was applied in 2012 and 2014 in the integrated treatment in the current trial and might potentially have reduced amino acid concentrations. In addition, high yields induce higher N uptake by the vines and can potentially reduce N reserves in the long run (Verdenal *et al.*, 2021). This might be one reason why lower amino acid concentrations were observed under int management, although treatments showed similar contents of mineralised NO<sub>3</sub>-N in the soil within the growing season (Fig. S1). At the beginning and the end of the growing season, org and bd treatments showed higher amounts of NO<sub>3</sub>-N in topsoil, although 25 kg ha<sup>-1</sup> of N were added to the int plots as mineral fertilisers in 2012 and 2014, respectively. The good N supply of org and bd treatments without the use of external inputs together with lower yield levels might partially account for the higher amino acid concentrations in org and bd plots. The concentration and the share of proline in the current study were quite high, indicating climate-dependent advanced ripeness of berries in all treatments (Schaller, 2005). In the integrated treatment, the share of proline was even slightly higher compared to organic and biodynamic plots, respectively. This result is not in accordance with Verdenal *et al.* (2021), who recently found a negative correlation between high yielding conditions and proline concentration. The integrated treatment showed a higher ratio of ammonium-N to amino acid-N compared to organic and the biodynamic plots. Differences in the fertilisation strategy may account for it. There was no evidence that copper application in the current trial negatively affected the amino acid concentration of the organic and biodynamic plots (Martins *et al.*, 2014). No copper was used in the integrated treatment. Shading treatments positively affected amino acid concentration in bunches, but enhanced light exposure of organic and biodynamic bunches did not lead to substantial losses of amino acid concentrations in the current trial (Friedel *et al.*, 2015).

The reduction of yield levels went along with a significantly lower number of clusters in the bunch zone of organic and biodynamic treatments. The reduced number of clusters in the bunch zone in organic and biodynamic plots did not lead to a higher number of exposed bunches but a lower number of shaded bunches and a higher share of exposed bunches. Berry skins of organic and biodynamic treatments subsequently showed a significantly higher amount of total flavonols and a different profile of hydroxycinnamic acids.

Flavanol concentrations did not differ among treatments. Friedel *et al.* (2015) described the strong influence of pre-bloom light interception in the bunch zone on flavanol composition. Therefore, it might be likely that differences in the canopy structure of the bunch zone among integrated, organic and biodynamic treatments might not be present at bloom but might develop later in the season due to lateral shoot growth. Döring *et al.* (2015) observed that lateral shoot growth differs significantly among integrated, organic and biodynamic management, with the integrated plots showing significantly higher lateral leaf area. Therefore, it might be likely that differences in bunch exposure among treatments might develop mainly in the second half of the growing season and might not have a major influence on flavanol composition.

Results on flavanols in the different management systems in the current study are in accordance with Tassoni *et al.* (2013); (Tassoni *et al.*, 2014) and Garaguso and Nardini (2015), who did not assess differences among management systems in berry flavanol concentrations and catechin concentrations in berries and wines. Tintunen and Lehtonen (2001), in contrast, found organic red wines from Burgundy to have significantly more flavanols compared to conventional red wines from Burgundy. Still, these results were obtained from commercial red wine samples and other factors might potentially impact their composition. Granato *et al.* (2015) report catechin and epicatechin concentrations in European juices from organic and biodynamic management to be higher compared to conventional juices, whereas Brazilian juices originating from different management practices did not differ in their catechin and epicatechin content in the same study. This shows that flavanol composition is highly linked to climatic and microclimatic conditions.

As flavanol synthesis, the synthesis of hydroxycinnamic acids takes place mainly before veraison. This is why Friedel *et al.* (2015) did not detect consistent differences in concentrations of hydroxycinnamic acids between leaf-removal and shading treatments at the fruit set. Still, in the current experiment, consistent differences in the composition of hydroxycinnamic acids were found. In contrast to Yıldırım *et al.* (2004), Granato *et al.* (2015) and Artem *et al.* (2021), which found juices and wines from organic and biodynamic management to have less coumaric acid, treatments in the current study did not differ in their concentration of coumaric acid in berry skins. Results are confirmed by Garaguso and Nardini (2015), who did not detect differences in coumaric acid in wines originating from different management practices. Still, the extractability of phenolic compounds and berry size might determine the final concentration of phenols in juices and wines. In the current study, treatments do not differ in their concentration of coumaric acid, but integrated plots show the highest concentrations of coumaric acid, whereas biodynamic plots show the highest concentrations of caffeic acid. This might indicate that within the phenolic biosynthesis pathway, more caffeic acid might be produced out of coumaric acid in the biodynamic treatment, whereas more coumaric acid might be produced in

the integrated treatment out of coumaric acid (Adams, 2006). Brandt *et al.* (2019) also showed that late defoliation led to significantly lower amounts of coumaric acid in berry skins of *Vitis vinifera* L. cv. Riesling. Rootstocks in the current study differed in their concentration of coumaric acid, with the more vigorous SO4 showing higher concentrations compared to Börner. Furthermore, the integrated treatment also showed significantly higher amounts of coumaroylglucose in the current study. Results are again supported by Brandt *et al.* (2019), who detected higher amounts of it in shaded compared to leaf-removal bunches. These might be hints that the phenolic biosynthesis pathway might be affected by differing management systems. Still, it cannot be cleared up to which extent results are solely determined by the differences in vigour and yield. To the best of our knowledge, this is the first detailed report of hydroxycinnamic acid composition in berry skins of white grapes from different management systems.

Flavonol accumulation takes place mostly post-veraison and has been shown to be highly light-dependent (Friedel *et al.*, 2015). Differences in the share of exposed clusters among treatments might play a major role in determining differences in flavonol concentrations in the respective treatments. Whereas Friedel *et al.* (2015) did not observe differences in que-3-glucuronide among leaf removal and shading treatments, biodynamic treatments showed significantly higher amounts of the latter. Brandt *et al.* (2019) showed que-3-glucoside as well as que-3-rhamnoside to be less abundant in shaded bunches. This is in accordance with the results from the current trial, where the integrated treatment showed significantly lower concentrations of both compounds together with a higher share of shaded bunches. Malusà *et al.* (2004) found flavonoid content in berry skins of organically managed plots to be significantly higher compared to conventional plots in a field trial on *Vitis vinifera* L. cv. Grignolino, although flavonoid content in red wines is highly linked to anthocyanins, which are not present in white wines. Vrček *et al.* (2011) also detected significantly higher concentrations of flavonols in red wines from Croatia originating from organic compared to conventional management. It would be of interest to study flavonol composition in shaded and exposed clusters from the respective management systems to unravel the extent to which bunch exposure determines flavonol content under integrated, organic and biodynamic viticulture. Rootstocks in the current study differed in que-3-galactoside, que-3-xyloside and que-3-arabinoside. These parameters were not influenced by the treatment itself. The rootstock had a higher influence on flavonols and limited to no influence on hydroxycinnamic acids and flavanols. Still, the function of the specific quercetin glycosides analysed in the current study is not yet fully understood and is likely to underlie strong developmental regulation.

The temperature in the bunch zone assessed during the growing season from 2012 to 2014 did not differ substantially among treatments (data not shown). Treatments did not differ significantly in their pre-dawn water potential when assessed

between 2012 and 2014. It can therefore be deduced that differences in hydroxycinnamic acid profiles and flavonol profiles among treatments are mainly an effect of the different light exposure of bunches rather than a temperature or a water stress effect.

A strong negative correlation between the accumulation of amino acids and phenolic compounds is observed due to the tight relation of these signalling pathways (Friedel *et al.*, 2015). Still, in the current study, a negative correlation between amino acid concentrations and flavonoids and hydroxycinnamic acids could not be observed. This might be due to the different influencing factors overlapping each other in their effects. Differences in yield seem to be primarily responsible for changes in amino acid concentration, whereas differences in light exposure of bunches, especially post-veraison, seem to influence flavonol composition in the current study. These two influencing factors are related as yield levels are coupled to light exposure of bunches under different management systems.

## CONCLUSION

Organic and biodynamic management was shown to highly impact yield, pruning weight, canopy structure, as well as amino acid composition in juice, flavonol and hydroxycinnamic acid composition in berry skins, compared to integrated management in a long-term field trial starting seven years after conversion. Organic and biodynamic management showed reduced yields and reduced pruning weights. These two factors seem to mostly account for significantly higher amino acid concentrations in organic and biodynamic plots. Applying synthetic fungicides in the integrated treatment might further decrease amino acid concentrations under integrated viticulture. Flavonol composition did not seem to be affected by the different management regimes, whereas the concentration of total flavonols was highly impacted by management. Total flavonol concentrations were significantly higher under organic and biodynamic management. These differences went along with a higher share of exposed bunches under organic and biodynamic treatments, which is likely to account for the observed differences. There is further evidence that the management system might impact the phenolic biosynthesis pathway concerning different hydroxycinnamic acids because differences in the composition of hydroxycinnamic acids were detected for the different management regimes, which are partially in accordance with other studies comparing shaded and leaf-removal bunches. Still, the function of the single hydroxycinnamic acids and flavonols is difficult to determine and further research is needed to unravel their role within grapevine berries.

Differences in yield and pruning weight and thus differences in the architecture of the bunch zone seem to be major drivers for the compositional changes observed under integrated, organic and biodynamic management reported here. It should be further investigated how differences in physiological



activity, root growth and nutrient availability determine grapevine performance in the respective treatments.

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