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Long-Term Industrial-Scale Study on Regulated Deficit Irrigation's Effect on Flavonoid and Volatile Composition in Cabernet Sauvignon (*Vitis Vinifera* L.) Grapes

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ABSTRACT

Background and Aims: Climate change and reduced precipitation pose significant challenges for the wine industry, particularly in vulnerable regions, where it is essential to incorporate irrigation management tools. Regulated deficit irrigation (RDI) is a step forward in that direction. Still, its adoption in Chile has been slow, partly due to inconsistent results regarding its impact on grape composition, a product of low experimental scalability, and, in general, highly variable applied water and vine stress due to the lack of actual evapotranspiration (ETa) assessment on study sites. This study aimed to understand the impact of RDI on yield, vine balance, and grape volatile and nonvolatile composition in an industrial-scale 5-year trial using precise ETa-based irrigation.

Methods and Results: A five-year experiment with three RDI regimes and a control was conducted in Maule Valley, Chile. Different portions of ETa from pea size to harvest were replenished as follows: 100% ETa (control), 70% ETa, 50%–100% ETa, and 35%–100% ETa (50 or 35% ETa before *veraison* and 100% ETa after). The experiment covered 4.54 ha, including a 1.2 ha control treatment in which a flux tower was installed to assess ETa. Midday stem water potential, yield components, and grape volatile and phenolic composition were measured. RDI reduced yield, mainly due to lower cluster weight as a result of smaller and fewer berries. Concentration on a fresh-weight basis of total anthocyanin, tri-hydroxylated anthocyanins, tannins, and specific flavonols, including quercetin, myricetin, and syringetin glycosides, was higher in the two most water-restrictive RDI treatments, while total soluble solids and acidity were lower. Also, the same treatments showed increases in positive aromas, including α -terpineol, β -damascenone, linalool, and nerol, as well as possibly undesired aromas such as IBMP and hexenal. While some metabolites indeed increased their per-berry content, suggesting an upregulation of metabolism, most of the observed increments were simply driven by berry size reduction.

Conclusions: Overall, most of the changes caused by RDI are positive in terms of grape quality, with higher color and aroma concentrations. To achieve most of the positive effects, RDI needs to reach at least 50% of the ETa, particularly from pea size to *veraison*.

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Significance of the Study: By incorporating actual ETa assessment in a 5-year industrial-scale trial, which enabled consistent irrigation fractions across seasons and reduced the uncertainty from crop-coefficient-based estimates, this study helped both to improve the reliability of RDI research and to address previous inconsistencies in RDI effects on grape chemical composition.

1 | Introduction

Due to the climate change scenario, water shortage is becoming the main limitation to the sustainability of Chilean viticulture. In this regard, irrigation management strategies, such as regulated deficit irrigation (RDI), are key to improve water productivity and to optimize grape yield and quality [1–5]. Although a large set of information regarding the impacts of RDI on yield components and grape composition has been published, the adoption of this irrigation strategy in Chile has been limited. This is the case in vineyards where the potential positive changes in wine composition might not compensate for the reductions in yield. Furthermore, the studies published in this area exhibit some inconsistencies, which can be attributed to seasonal variability, the small size of the trials, and the inherent differences among treatments based on incomplete evapotranspiration replenishment due to the use of different crop coefficients and the lack of evapotranspiration measurements on the site. Like so, some trials have only focused on phenolics or aroma effects, but not on both, making it difficult to understand the overall impact of RDI on grape and wine quality. Here, we address this gap by applying continuous in-field ETa monitoring across an industrial-scale trial, allowing precise quantification of applied water fractions and interseasonal comparability.

In general, water deficit has been shown to modify the morphological, physical, and chemical characteristics of berries, including pH, titratable acidity, sugar, phenolic content, and aromas [6–9]. In addition, water stress can limit vegetative development, increasing the exposure of grape clusters to solar radiation, which also impacts their chemical composition [1, 10]. This condition may result in thicker berry skins, higher phenolic content, reduced water accumulation, and a corresponding increase in the concentration of soluble solids in the grapes. Finally, vines that are grown in soils with lower water availability will generate smaller berries than those growing with an adequate water supply [11–18].

The physiological cell growth functions affected in vines growing under low water availability will depend on the phenological stage at which the deficit irrigation occurs. When water deficit happens between fruit set and *veraison*, a smaller berry size is observed, which is primarily attributed to decreased cell division [19]. Instead, when the water deficit occurs from *veraison* to preharvest, the reduction in berry size is due to limited cell growth and water accumulation, with a less severe impact on final berry size compared to a water restriction near fruit set [20]. These two effects (i.e., reduced cell division and limited cell growth), which occur simultaneously when the vineyards are subjected to the RDI, may contribute to an increased concentration of metabolites of winemaking interest, such as anthocyanins and flavonols [11]. However, this may also happen due to changes at the metabolic level, by increased synthesis, or by changes in the degradation of these metabolites [21–24], some of which are mediated by abscisic acid, which has been reported to increase under water deficit [25, 26].

Water deficit affects the chemical composition of red grapes and wines, resulting in increased concentration of total anthocyanins [21, 27–33], total phenols [34], total tannins, polymeric pigments [27, 35], and the mean degree of tannin polymerization [6, 31, 36], increases flavonols, hydroxycinnamic acids [31, 37], as well as the flavan-3-ol polymeric concentration in grape skins [6]. However, a decrease in flavonol concentration under water deficit treatments has also been reported by [30]. Alternatively, the authors of [31, 32, 38] reported no differences in total flavan-3-ols and flavonols in certain years, while the authors of [39, 40] found no differences in total tannin concentration. Theodorou et al. [41] also reported discrepancies in the behavior of total anthocyanins among different red varieties.

Some of the metabolites reported are also stimulated by radiation [42, 43] as an indirect effect of water deficit. The control of vegetative growth observed in vines under water deficit considerably increases the solar radiation interception by grapes [10, 29, 34, 44] and the temperature at which the clusters are exposed during their development [29, 44]. The relationship between the increase in anthocyanins and total phenols with the reduction in vigor and a greater solar interception in the cluster zone of vines subjected to deficit irrigation has also been reported, but further research is needed to draw reliable conclusions [29, 34].

Regarding fruit/wine aroma composition, RDI has been shown to have a positive impact on aroma quality by enhancing fruity aromas of red wines. However, data on this topic are limited, and conclusions remain unclear for some high-impact aroma compounds [31, 45–47]. For example, Talaverano et al. [8] reported that water deficits increased volatile acids and reduced the concentration of esters in Cabernet Sauvignon grapes. However, the study also reported that the effects on aromas were strongly influenced by harvest date and seasonal variations, possibly having a greater impact than water stress. This suggests that water deficit treatments might have minimal to no effects on aroma if the harvest is delayed and higher physiological maturity is achieved. Regarding positive floral and fruity aromas, such as β -damascenone and β -ionone, some authors reported increased concentrations in fruit grown under RDI treatments [40, 48] while others indicated no effect [31, 49]. In a study conducted with Cabernet Sauvignon in Spain, García-Esparza et al. [36] showed that deficit irrigation, as well as dry-farmed rain-fed treatments, has a considerably lower concentration of aldehydes such as hexanal, known to have an undesirable impact on green aromas (mowed grass), thus having a positive impact on wine aroma quality. Alternatively, Méndez-Costabel et al. [46] did not find differences in any of these volatiles.

RDI has been shown to reduce the vegetal aroma profile in wines related to pyrazines, such as isobutyl-methoxypyrazine [45], the compound responsible for the bell pepper aroma in wines, which, in high concentrations, might be undesirable to some

winemakers. However, these changes do not necessarily correlate with the sensory perception of vegetal aromas of the wines, as reported by Mendez-Costabel et al. [46]. Additionally, light exposure is known to reduce the synthesis and concentration of methoxypyrazines [50, 51]; therefore, deficit irrigation treatments that reduce canopy growth and increase overall cluster exposure might affect the concentration of this compound.

Although numerous RDI experiments have been conducted, the effects of irrigation treatments on grape yield and the chemical characteristics of berries have shown positive, negative, or neutral results. These responses to RDI treatments are influenced by complex, nonlinear interactions between factors such as cultivar, soil, climate variability, and agronomic practices, including fertilization, pruning, and irrigation.

Even though many of these trials were done on the same cultivar, with equivalent levels of deficit irrigation, the treatments are far from being similar in terms of irrigation applied and level of stress reached by the plants. These fundamental differences on the level of stress experienced by the vines have to do, at least in part, with the fact that no evapotranspiration measurements were made in those trials, and most of them depended on the use of different crop coefficients (K_c) from bibliography, which only give an estimation of the irrigation needed to completely supply the ETa or partial ETa for RDI treatments. Research focusing on the long-term effects of these interactions on the biochemical characteristics of berries is essential for optimizing water use, yield, and quality in vineyards grown under Mediterranean climate conditions. Therefore, the aim of this study was to evaluate the long-term effects of RDI on yield components and grape chemical composition (phenolic, anthocyanin, and aroma profiles) of drip-irrigated Cabernet Sauvignon vines growing under Mediterranean climate conditions, where precise ETa monitoring is in place, in order to standardize RDI treatments and reduce variability in the response derived from irrigation.

2 | Materials and Methods

2.1 | Experimental Site Description

A five-year RDI experiment was conducted in a commercial vineyard (cv. Cabernet Sauvignon) located in the Penciahue Valley (-35.26°S , -71.48°W ; 105 m above sea level), Maule Region, Chile. This valley has a temperate of Mediterranean climate with an annual rainfall of 527 mm (1991–2020 Talca weather station) [52] mm year^{-1} and a reference evapotranspiration (ETo) of 1324 mm [53]. The growing seasons (from November to April) are dry and hot, with an average daily temperature ranging from 10.7°C to 29.3°C and ETo ranging from 3.1 to 6.1 mm day^{-1} [53]. The vineyard soil is a sandy loam of medium depth (90–150 cm) over a cemented hardpan.

The vine material was Cabernet Sauvignon clone 341 grafted on SO4 and planted in 2014, trained into a vertical shoot position (VSP) trellis system and cane pruned (double guyot) with a vine spacing of 1.2 m within rows and 2.3 m between rows, in a north-east to south-west orientation. The vineyard was drip-irrigated with pressure-compensated emitters (flow rate $\approx 2.5 \text{ L/h}$) spaced 0.6 m apart. The source was river water from the Andes snowpack with an average EC of 0.3 dS/m .

2.2 | Experimental Design

Four treatments were applied in a completely randomized block design, using soil as a blocked factor. The soil was blocked into four areas due to differences in depth, with one replicate per treatment in each block, with a total of four replicates per treatment (Figure 1). The experimental unit consisted of six continuous rows of 120–190 m length, with a total surface area of 0.22–0.26 ha each, with the two center rows used as observational units (320 vines). The four treatments were defined as the percentage of replacement of actual evapotranspiration (ETa, mm day^{-1}) from flowering to harvest.

Treatments were coded as 100% ETa, 70% ETa, 50%–100% ETa, and 35%–100% ETa. For 100% ETa (control) and 70% ETa, irrigation was corresponded to 100% and 70% of the ETa during the entire growing season, respectively. For the 50%–100% ETa treatment, irrigation was based on 50% of the ETa from berry set (BS) to veraison (V) and 100% ETa from V to harvest (H). In the 35%–100% ETa treatment, irrigation was based on 35% of the ETa from BS to V and 100% from V to H.

Daily values of vine water consumption were estimated using the surface renewal (SR) technique which calculates ETa as a residual of the energy balance equation ($\text{ETa}\lambda = \text{LE} = \text{Rn} - \text{H} - \text{G}$) where λ is the latent heat of vaporization (MJ kg^{-1}), LE is the latent heat flux ($\text{MJ m}^{-2} \text{ s}^{-1}$), Rn is the net radiation ($\text{MJ m}^{-2} \text{ s}^{-1}$), H is the sensible heat flux ($\text{MJ m}^{-2} \text{ s}^{-1}$), and G is the soil heat flux ($\text{MJ m}^{-2} \text{ s}^{-1}$). In this technique, daily values of soil heat flux (G) are considered negligible [54–56], and sensible heat flux density (H) is estimated based on the classical structure-function analysis method of Van Atta. The setup includes a sonic anemometer to calibrate the estimates of (H) by Eddy Covariance. The flux tower was located in the control treatment at the center of the experiment, in an experimental unit of 1.2 ha of surface area, in order to have enough fetch to correctly assess evapotranspiration with this technique. Water was applied on

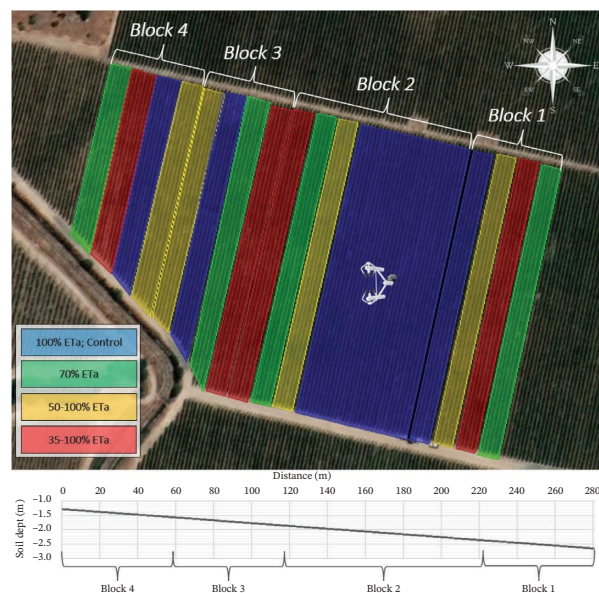


FIGURE 1 | Experimental design showing the location of the blocks. Each block had one repetition per treatment. Image also shows the location of the flux tower (white icon of a sonic 3D anemometer) to calculate ETa using surface renewal and eddy covariance.

a weekly basis, according to the ETa measurements of the previous week, and treatments were obtained by controlling the valves in each row in order to give 100%, 70%, 50%, or 35% of the ETa. To do this, all the irrigation lines were intervened by installing valves to control irrigation.

All fertilizers, herbicides, and pest management practices were applied following commercial standards with no difference between treatments.

2.3 | Plant Water Status

Stem water potential (Ψ_x , MPa) measurements were taken weekly at solar noon using a pressure chamber (Model 1000, PMS Instrument Company, Corvallis, OR, USA). Two mature, healthy, and undamaged leaves were randomly selected from different plants from the two center rows in each replicate (totaling eight leaves per treatment) from the middle third of the canopy. The leaves were wrapped in foil-laminate zip-lock bags (PMS Instrument Company, Corvallis, OR, USA) for at least 1 hour before the measurements were taken [5, 57].

2.4 | Climate Characterization

Characterization of the specific growing season was assessed by the total accumulated thermal time, using growing degree days (GDD) with the single sine method [58]. A Biofix was set from September 1st to April 30th. The base temperature and upper-temperature limit thresholds were 10°C and 35°C, respectively, as proposed by Ortega-Farias & Riveros-Burgos [59] as accurate developmental thresholds for grapevines. Temperatures were obtained from an automatic meteorological station (Adcon telemetry, A730, Klosterneuburg, Austria) located inside the property near the experimental site (2 km).

2.5 | Yield and Vine Balance

During harvest, the yield was calculated by dividing the total amount harvested in each experimental unit by the number of plants harvested and then multiplying by the number of plants per hectare. The same information was used to assess the Ravaz Index in winter, where all the vines from the observational unit (two center rows) were pruned (320 vines), and then, the pruning material of six continuous vines was packaged and weighed. This was repeated fifteen times in each replicate. The Ravaz Index was then calculated by dividing the average values of plant yield by the average pruning weight per plant.

2.6 | Sampling at Harvest

At technical ripening, defined as 23.5 °Brix, a sample of approximately 1000 kg of fruit from each experimental unit was hand-harvested in 500 kg bins to assess yield, must composition, and to be later vinified. Also, a subsample of fifty clusters in each replicate was collected to assess grape and cluster physical characteristics and grape physiochemical composition, including cluster weight, berry weight, number of berries per cluster, and grape phenolic and aroma composition. This subsample of complete clusters was randomly taken from both sides of the canopy (i.e., east and west), with 25 clusters per side, in a constant selection pattern of one cluster located inside and another located outside the vine canopy (i.e., shaded and exposed to direct sunlight). The harvest date for each treatment was

determined independently, based on when the grapes reached 23.5 °Brix.

2.7 | Laboratory Methodologies

Most of the analytical methods listed below have been previously described [32, 60, 61] and are summarized below.

2.7.1 | Grape and Clusters Physical Characteristics

The 50 clusters were individually weighed. Then, berries were destemmed, counted, and weighed. The final data collected were cluster weight, number of berries per cluster, berry weight, and stem weight.

2.7.2 | Grape Chemical Analyses

2.7.2.1 | Basic Must Analyses. A juice sample was taken from the 1-ton tank described in 2.6 after destemming and crushing, following 5 minutes of mechanized punch-downs to ensure grape-must homogeneity. Total acidity (TA), pH, and total soluble solids (TSS) (°Brix) were assessed by standard methodologies from the Organisation Internationale de la Vigne et du Vin (OIV) [62]. Malic acid and primary amino nitrogen (PAN), rapid ammonia nitrogen (RAN), and yeast assimilable nitrogen (YAN) were spectrophotometrically analyzed with enzymatic kits (K-DMAL, K-AMIAR, and K-NOPA enzymatic kits, respectively) purchased from Megazyme (Wicklow, Ireland). References and data booklet are available at <https://www.megazyme.com>.

2.7.2.2 | Grape Chromatography Analyses. Prior to analysis, grape samples were preconditioned as follows: berries of the 50-cluster subsample per replication were turned into a homogenate by using a blender for 30 s and 1 min with a handheld blender. The sample was then frozen and stored at −20°C for analysis.

2.7.2.2.1 | Analysis of Volatile Compounds by GC-MS/MS. Methoxypyrazines, norisoprenoids, terpenes, C6 aldehydes and alcohols, and esters were extracted with headspace solid-phase microextraction (SPME) and analyzed with gas chromatography with tandem mass spectrometry (GC-MS/MS, 7890B GC oven, 7000C Triple Quad, with a 7693 ALS CombiPal 7697A Headspace sampler, all Agilent Technologies Santa Clara, USA), using a DB Wax 30 m × 0.25 mm × 0.25 μm capillary column (Agilent, Santa Clara, USA) and helium as a carrier gas. SPME sampling was done with divinylbenzene polydimethylsiloxane fiber (PDMS-DVB) 65 μm (Supelco, Bellefonte, USA). The following two methodologies were used as references with minor modifications associated with equipment and optimization tests: (a) methoxypyrazines, 3-isobutyl-2-methoxypyrazine (IBMP), and 3-isopropyl-2-methoxypyrazine (IPMP) were analyzed based on [63] and (b) norisoprenoids; β-damascenone, β-ionone, terpenes; 1,4-cineole, 1,8-cineole, linalool, α-terpineol, citronellol, nerol, geraniol, and C6 aldehydes; hexanal, trans-2-hexenol, cis-3-hexenol, trans-3-hexenol, benzaldehyde, and benzyl alcohol, as free and glycosylated precursors, based on the methodology of [64]. The reference standards used are listed in Table 1 of the Supporting Information.

TABLE 1 | Total irrigation applied per treatment over the five consecutive growing seasons and water productivity.

Treatment		100% ETa	70% ETa	50%–100% ETa	35%–100% ETa	P-value treatment
Irrigation (m ³ /ha)	2017–2018	3566	2504	2423	1904	ND
	2018–2019	4310	3175	3114	2496	ND
	2019–2020	5637	4272	4169	3743	ND
	2020–2021	5289	3827	3784	3464	ND
	2021–2022	4442	3293	3222	2827	ND
	Average	4690	3438	3366	2899	ND
Water productivity (kg fruit/m ³)	2017–2018	4.53 ± 0.13 c	6.18 ± 0.48 ab	4.87 ± 0.20 bc	6.29 ± 0.17 a	< 0.0022
	2018–2019	4.48 ± 0.03 c	5.60 ± 0.40 ab	4.83 ± 0.40 bc	5.87 ± 0.21 a	< 0.0425
	2019–2020	2.82 ± 0.11	3.40 ± 0.24	2.85 ± 0.17	3.22 ± 0.17	ns
	2020–2021	3.15 ± 0.13 b	4.31 ± 0.39 a	3.57 ± 0.17 ab	3.98 ± 0.19 ab	< 0.0308
	2021–2022	3.53 ± 0.27 b	4.87 ± 0.40 a	4.32 ± 0.21 ab	4.54 ± 0.25 ab	< 0.0368
	Average	3.70 ± 0.17 b	4.87 ± 0.28 a	4.11 ± 0.21 ab	4.78 ± 0.27 a	< 0.0019

Note: Different lowercase letters in horizontal order indicate significant differences between the treatments in the different seasons p -value ≤ 0.05 , Tukey HDS test.

Grape Extraction for Aromas: 80 g of the frozen grape homogenate was defrosted and mixed with 80 mL of citrate buffer at pH 6 and then homogenized with an Ultra Turrax T25 (Ika, Staufen, Germany) at 15000 rpm for one minute. Then, the sample was shaken with an orbital shaker (Jeio Tech, model OS-2000, Daejeon, Korea) for 2 h and centrifuged for 10 min at 4000 rpm. The supernatant was separated and used directly to analyze free and hydrolyzed aromas (in the case of glycosylated aromas).

Direct Analysis:

- **Methoxy pyrazines:** in a 20 mL vial for SPME, 8 mL of the supernatant was mixed with 3 g of NaCl (standards are also added in case of validation and recuperation rate assessments). The vial was then incubated at 35°C with the SPME fiber exposed for 15 min, followed by desorption at 240°C, and injected in splitless mode into the GC.

Hydrolysis:

- **Hydrolysis for C6 compounds and terpenes (linalool):** In a 20 mL vial for SPME, 8 mL of the supernatant was mixed with 3 g of NaCl and 0.1 mL of sulfuric acid (standards are also added in case of validation and recuperation rate assessments). Then, the sample was incubated at 40°C for 15 min and exposed to the SPME fiber. The sample was later desorbed at 250°C and injected in splitless mode into the GC.
- **Hydrolysis for cineole, terpenes (α terpineol, citronellol, nerol, geraniol) norisoprenoids, benzene derivatives, and alcohols:** in the 20 mL SPME vial, 8 mL of the supernatant was mixed with 3 g of NaCl and 0.45 mL of sulfuric acid (standards are added also in case of validation and recuperation rate assessment). Then, the vial was incubated at 75°C for 15 min with the SPME fiber, which was then desorbed at 250°C and injected into the GC in a splitless mode.

2.7.2.2.2 | Analysis of Polyphenols by HPLC-DAD.

Grape extraction for polyphenolic analysis: 50 g of the grape homogenate was mixed with 50 mL of hydrochloric acid (0.1 N with ethanol, 70:30 v/v), homogenized with an Ultra Turrax, and shaken and centrifuged under the same conditions as for aroma analysis in order to obtain a supernatant as the sample to be analyzed.

Monomeric anthocyanins: Analyses were performed in an HPLC-DAD, 1290 Infinitely Series, 1260 DAD G4212B, oven 1290 TCC G1316C (Agilent Technologies Santa Clara, USA), using a Zorbax SB C18 column, 150 × 2.1 mm, 5 microns (Agilent Technologies Santa Clara, USA), and two mobile phases of water/formic acid/ acetonitrile (87:10:3 by vol.) for phase A and (40:10:50 by vol.) for phase B. A reference standard of malvidin-3-glucoside (CAS: 7228-078-6, from Extrasynthese (Genay, France) was employed for quantification, and identification was based on elution order and retention time, based on the reference methodology proposed by the OIV [65].

Supernatant samples were filtered with 13 mm × 0.22 μ m Millex filter. 2 mL of the filtered sample was directly injected onto the HPLC and separated with the following solvent gradient: phase A started with 96% in time 0 and moved to 70% after 6 min, 60% after 13 min, 55% after 15 min, 70% after 18 min, and back to 96% after 23 min.

Anthocyanins measured were delphinidin-3-O-glucoside, cyanidin-3-O-glucoside, petunidin-3-O-glucoside, peonidin-3-O-glucoside, malvidin-3-O-glucoside, peonidin-3-O-acetyl-glucoside, malvidin-3-O-acetyl-glucoside, peonidin-3-O-coumaroyl-glucoside, and malvidin-3-O-coumaroyl-glucoside.

Low molecular weight polyphenols (LMWPs): LMWPs were analyzed in a liquid chromatograph (same as for anthocyanins), fitted with a Purospher STAR RP-18 column (250 × 4.6 mm, 5 microns; Meck, Darmstadt, Germany). The same two mobile phases and protocol to obtain the supernatant described for anthocyanins were used in this case.

5 mL aliquot of the grape sample supernatant was mixed with 5 mL of hydrochloric acid 0.1 N and ethanol (70:30 by vol) solution and loaded in a solid-phase extraction (SPE) polymeric cation exchange column SPE (Bond Elut Plexa PCX 500 mg, 6 mL; Agilent Technologies, Santa Clara, USA), previously activated with 5 mL of methanol and 5 mL of deionized water. Then, the column was washed with 5 mL of the hydrochloric acid/ethanol solution, followed by 10 mL of deionized water. Finally, the samples were eluted with 15 mL of methanol. The eluted samples were concentrated with a rotary evaporator HB10 (Ika, Staufen, Germany) at 36°C until total evaporation. After this, the samples were reconstituted with 5 mL of a solution of 98:

2 v/v of the mobile phase A and mobile phase B. In this case, the chromatographic gradient used was as follows: phase A started with 94% at time 0 and moved to 65% after 15 min, 55% after 30 min, 40% after 35 min, 20% after 40 min, and back to 40% after 45 min.

The detector was set to acquire information at $\lambda = 280$ nm for flavan-3-ols, $\lambda = 320$ nm for hydroxycinnamic acids, and $\lambda = 360$ nm for flavonols. The chromatographic flow rate employed was 0.5 mL/min.

Molecules measured include flavan-3-ols; catechin, epicatechin, epicatechin-gallate, flavonols; myricetin 3-O-galactoside, myricetin 3-O-glucoside, myricetin, quercetin 3-O-rutinoside (rutin), quercetin 3-O-galactoside (hypersoside), quercetin 3-O-glucoside, quercetin, laricitin hexoside, syringin 3-O-galactoside, syringin 3-O-glucoside, kaempferol, and phenolic acids; caftaric acid, caffeic acid, and coumaric acid.

When compounds were below LOQ, they were reported as < LOQ and excluded from statistical analysis.

The reference standards used for LMWP are listed in Table 2 of the Supporting Information.

2.7.2.3 | UV-Vis Spectroscopic Measurements. Acquisition of UV-Vis spectra (190–750 nm), Harbertson–Adams assay [66] for anthocyanin and tannins concentration, and total tannins by indirect determination through precipitation with methylcellulose [67] were analyzed.

2.8 | Statistical Analysis

Data were subjected to Shapiro–Wilk’s normality test and Levene’s test prior to further analysis. If the data did not pass these tests, transformations were used to achieve normality. Results were analyzed by multifactor ANOVA considering irrigation (4 levels) as the main factor and adding year (5 levels), and soil block (4 levels, one repetition per soil block). Both year and soil blocks were added as blocked factors. Standard error and mean were calculated. When the *p*-value was significant (*p*-value ≤ 0.05), a Tukey’s (HSD) multiple comparison test was used to compare

the means and define significant differences between treatments. All analyses were done using Statgraphics Centurion XVI software (StatPoint Technologies, Virginia, USA).

3 | Results

3.1 | Climate During the Study

All seasons studied began with similar GDD accumulation, but differences became noticeable from the end of January to mid-February (Figure 2), just when *veraison* was finished and irrigation was restored back to 100% on the 50%–100% ETa and 35%–100% ETa treatments. This means that most of the actual deficit irrigation in these treatments occurs under similar climatic conditions every year. From February to the end of April, the variations among seasons allowed differentiation between warmer and colder seasons. This was a period where the fruit remained on the vine, and the ripening process was actively happening, but where only the 70% ETa treatment had a different irrigation than the other treatments.

The lowest and highest GDD values were 1895°C in the 2021–2022 season and 2133°C in the 2019–2020 season, respectively, while the other seasons had intermediate conditions: 2020–2021 with 2020 GDD, 2017–2018 with 2032 GDD, and 2018–2019 with 2067 GDD. Additionally, no clear relationship was found between accumulated GDD and the harvest date. Although the 2021–2022 season was the coldest, it was the first to be harvested. Instead, the hottest season, 2019–2020, was the second earliest to be harvested. In this study, the harvest dates were March 12th (at 1757° GDD) in the 2019–2020 season, March 28th (at 1877° GDD) in the 2018–2019 season, March 30th (at 1842° GDD) in the 2020–2021 season, March 31st (at 1744° GDD) in the 2021–2022 season, and April 4th (at 1907° GDD) in the 2017–2018 season.

Another way to characterize the climate during the five seasons was by comparing their environmental water demand, calculated as Penman–Monteith reference evapotranspiration (ETo). Figure 3 shows that there were slight differences among growing

TABLE 2 | Effect of regulated deficit irrigation on yield and their components for a drip-irrigated vineyard (cv. Cabernet Sauvignon) over five consecutive growing seasons.

Treatment	Yield/ha (kg)	Pruning weight/plant (kg)	RAVAZ index	Cluster weight (g)	Berry weight (g)	Berries per cluster
100% ETa	16905 $\pm$ 382 a	1.04 $\pm$ 0.02 a	4.53 $\pm$ 0.18 ab	161 $\pm$ 2.5 a	1.17 $\pm$ 0.01 a	129 $\pm$ 1.92 a
70% ETa	15479 $\pm$ 476 b	0.84 $\pm$ 0.02 d	5.08 $\pm$ 0.29 a	142 $\pm$ 2.3 b	1.06 $\pm$ 0.01 b	125 $\pm$ 1.79 a
50%–100% ETa	13737 $\pm$ 487 c	0.94 $\pm$ 0.02 b	4.08 $\pm$ 0.08 b	119 $\pm$ 1.8 c	0.97 $\pm$ 0.01 c	115 $\pm$ 1.57 b
35%–100% ETa	13069 $\pm$ 337 c	0.89 $\pm$ 0.02 c	4.16 $\pm$ 0.15 b	117 $\pm$ 1.9 c	0.93 $\pm$ 0.01 d	117 $\pm$ 1.64 b
P-value treatment	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
2017–2018	13978 $\pm$ 387 B	0.93 $\pm$ 0.02 C	4.25 $\pm$ 0.18	133 $\pm$ 2.9 C	0.94 $\pm$ 0.01 D	130 $\pm$ 2.48 A
2018–2019	16348 $\pm$ 418 A	1.08 $\pm$ 0.02 A	4.33 $\pm$ 0.18	159 $\pm$ 2.7 A	1.18 $\pm$ 0.00 A	125 $\pm$ 2.20 B
2019–2020	14203 $\pm$ 402 B	0.79 $\pm$ 0.02 D	4.65 $\pm$ 0.18	118 $\pm$ 3.0 D	0.96 $\pm$ 0.01 D	117 $\pm$ 2.50 C
2020–2021	14861 $\pm$ 402 AB	1.00 $\pm$ 0.01 B	4.24 $\pm$ 0.18	150 $\pm$ 3.0 B	1.09 $\pm$ 0.01 B	132 $\pm$ 2.49 A
2021–2022	14597 $\pm$ 402 B	0.84 $\pm$ 0.01 D	4.83 $\pm$ 0.18	112 $\pm$ 3.0 D	1.00 $\pm$ 0.01 C	104 $\pm$ 2.49 D
P-value season	0.001	< 0.001	0.0803	< 0.001	< 0.001	< 0.001
P-value T x S	0.874	0.121	0.967	< 0.001	< 0.001	< 0.001

Note: Different lowercase letters in vertical order indicate significant differences between the treatments, and different uppercase letters in vertical order indicate significant differences between season *p*-value ≤ 0.05 , Tukey HDS test.

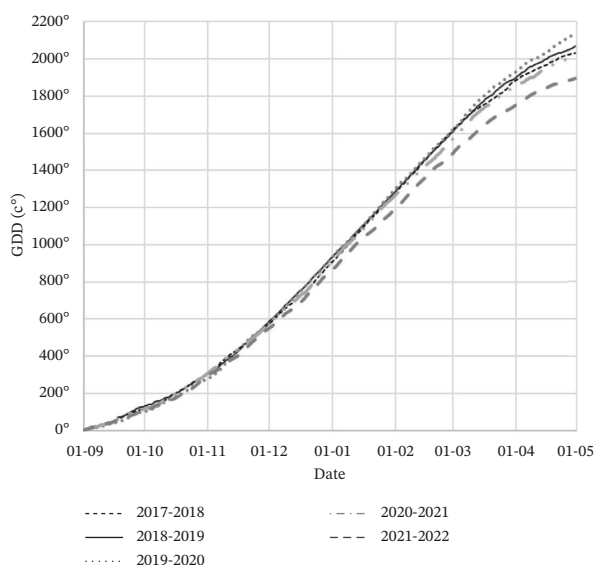


FIGURE 2 | Comparison between the growing degree days calculated in single sine with fixed dates between 01-09 and 30-04 and temperature thresholds of 10°C and 35°C as min and max.

seasons and that the main differences started to be noticeable in the middle of them. Seasons 2018–2019 and 2020–2021 had a few weeks in the middle where environmental demand was considerably lower, which was associated with some rain events occurring during the summer. The opposite happened with the 2019–2020 season, which had a higher environmental demand from the middle to the end. The overall accumulated evapotranspiration from September to April gives a good idea of how demanding each season was. In this case, the seasons with the lowest to the highest demand were as follows: 2017–2018 with 1051 mm, 2021–2022 with 1058, 2018–2019 with 1090, 2020–2021 with 1116 mm, and 2019–2020 with 1228 mm.

Considering both climate indicators (i.e., GDD and ETo), a clear idea of the weather conditions of the most extreme seasons could

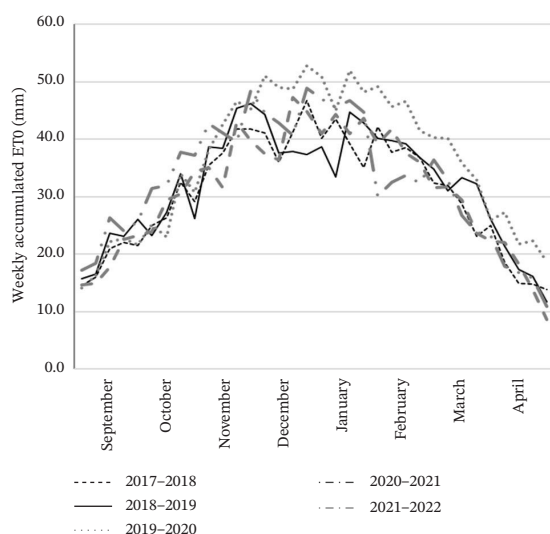


FIGURE 3 | Comparison between the water environmental demand calculated as ETo by Penman–Monteith equation of the five different seasons.

be achieved, with a warm and high water-demanding 2019–2020 and a cold, low water-demanding 2021–2022. The other seasons did not show a clear relationship with these two indicators, and they rank between 2019–2020 and 2021–2022 in terms of temperature and water demand.

3.2 | Water Status and Water Use

Figure 4 shows that there were significant differences in midday stem water potential (Ψ_{md}) between RDI treatments and the control during the five growing seasons. The level of these differences varied among seasons, but the following general behavior was observed: once RDI was applied, all treatments showed a reduction in Ψ_{md} compared to the control, becoming significantly lower after two weeks and, in some years, even earlier. Treatments 35%–100% ETa and 50%–100% ETa exhibited a similar behavior, becoming statistically lower than 70% ETa three to four weeks after the RDI began. The difference between 35% and 100% ETa and 50%–100% ETa typically appeared seven to eight weeks after RDI started and lasted for a brief time. After the RDI period of 35%–100% ETa and 50%–100% ETa, when irrigation was restored to 100%, the 70% ETa became the most stressed treatment, since it stayed under RDI until harvest. Instead, 35%–100% ETa and 50%–100% ETa rapidly recovered and reached values equivalent to the control.

The control treatment maintained values between -0.43 and -1.19 MPa in the 2018–2019 season. This season was the one with the biggest amplitude, along with the 2020–2021 season, reaching a Ψ_{md} of -1.19 MPa. The lowest values for all treatments were observed between 60 and 80 days after flowering (DAF) during January, just prior to full *veraison*, where the environmental demand expressed as ETo reached its peak demand (Figure 3).

Regarding water use, the average irrigation volume for the control treatment was $4690 \text{ m}^3/\text{ha}$, with considerable variation between seasons, ranging from $3566 \text{ m}^3/\text{ha}$ in 2017–2018 to $5637 \text{ m}^3/\text{ha}$ in 2019–2020 (Table 1). This variation was directly associated with reference evapotranspiration, with 2017–2018 having the lowest ETo levels and 2019–2020 the highest. The average reduction in total irrigation water use was 26.7% for the 70% ETa treatment, 28.2% for the 50%–100% ETa treatment, and 38.2% for the 35%–100% ETa treatment. Notably, the 2017–2018 season, characterized by low ETo, showed the highest water savings: 29.8%, 32.0%, and 46.7% for the 70% ETa, 50%–100% ETa, and 35%–100% ETa treatments, respectively. In contrast, the 2019–2020 season, with high ETo, showed the lowest water savings: 24.2%, 26.0%, and 33.6% for the same treatments, respectively.

Water productivity was improved by some RDI treatments, although no linear relationship was found between the level of irrigation restriction and the total water saved against water productivity. The two treatments that showed the highest average water productivity were 70% ETa and 35%–100% ETa, increasing fruit production per cubic meter of water used by 31.6% and 29.2%, respectively. The 50%–100% ETa treatment did not improve water productivity significantly against the control, although its performance was also not statistically different from the other RDI treatments.

On a yearly basis, it is noteworthy that the low water-demanding 2017–2018 season exhibited the greatest differences in water

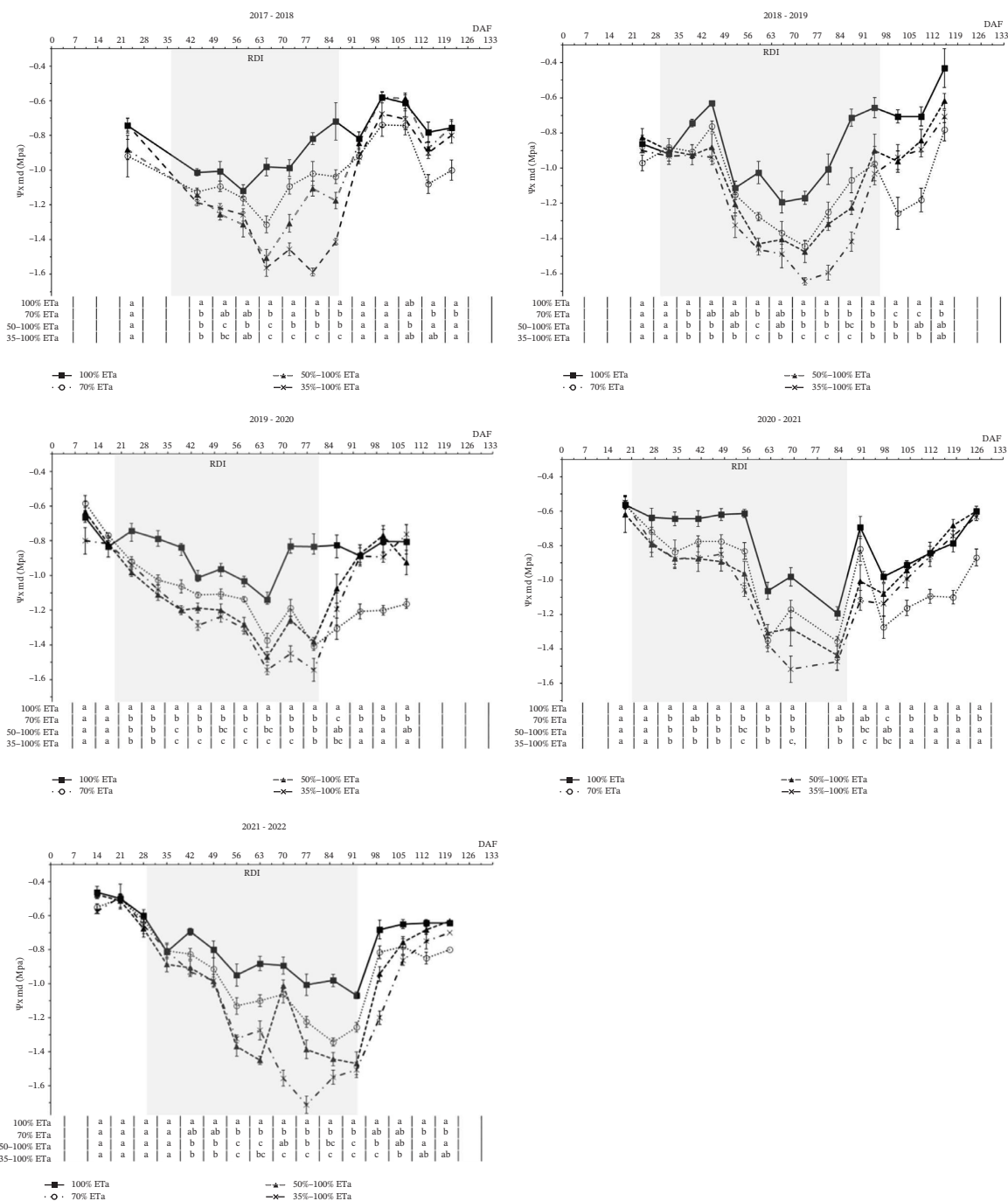


FIGURE 4 | Water status of the treatments during the five experimental seasons measured as midday stem water potential ($\Psi_{x\ md}$). 100% ETa, 70% ETa, 35%-100% ETa, and 50%-100% ETa. Days after flowering (DAF).

productivity between the control and the 70% ETa and the 35%-100% ETa treatments. Conversely, the high water-demanding 2019-2020 season showed no significant differences between the treatments and the control. Additionally, across seasons, the 70% ETa treatment consistently demonstrated improved water productivity.

3.3 | Yield and Its Components

Table 2 shows significant effects of RDI treatments on fruit yield and productivity components. In this study, the final yield was directly correlated with the amount of irrigation applied, with

more severe restrictions showing significant yield reductions. Although the differences observed between treatments varied among seasons (i.e., 2018-2019 being larger, and 2017-2018 smaller, data not shown), the final impact of RDI was equivalent over the 5 years studied. For the 35%-100% ETa and 50%-100% ETa treatments, the yields were 22.7% and 18.7% lower than the control, respectively, with no statistically significant difference between them. For the 70% ETa treatment, the yield was 8.4% lower than the control. The RDI effect on yield was largely associated with reductions in the cluster weight, which was a product of lighter and fewer berries per cluster. Treatments 70% ETa, 50%-100% ETa, and 35%-100% ETa had significantly lower

cluster weights than those in the control, but no differences were found between 50% and 100% ETa and 35%–100% ETa. Regarding berry weight, all treatments were significantly different, showing that the more severe the deficit irrigation, the lighter the berries produced. The number of berries per cluster was similar between the control and 70% ETa, which were statistically greater than those of 50%–100% ETa and 35%–100% ETa. When looking at the effects on vine balance and vigor, it was possible to confirm that RDI treatments affected pruning weight, showing almost linear reductions with increasing water restrictions. The prior happened except for the 70% ETa treatment, which was the only one where water restriction was maintained until harvest, having the lowest pruning weight. When observing the impact on vine balance (Ravaz), the control treatment did not have any difference with any of the RDI treatments; nevertheless, 70% ETa was significantly higher than 50%–100% ETa and 35%–100% ETa treatments. This means that although the RDI treatments reduced yield and pruning weight, the reductions maintained the same fruit-to-canopy ratio as the control treatment. However, when RDI is imposed, maintaining a constant deficit such as 70% ETa will increase the Ravaz ratio against the RDI treatments that recover the irrigation after veraison, such as 50%–100% ETa and 35%–100% ETa, resulting in a higher yield per pruning weight.

Regarding the effects of the season's climate, Table 2 shows how cluster and berry weight are lower in the 2019–2020 season, the warmest and most water-demanding one, being also a season with low yield and pruning weight. On the other hand, cold and low water-demanding seasons like 2021–2022 also had the lowest cluster weight, but this time, it is accompanied by the lowest number of berries per cluster. Interactions between the irrigation treatment and season were only significant for cluster weight, berry weight, and berries per cluster. These interactions are mainly driven by a reduction of the level of differences observed between treatments in a few seasons, detailed in the Supporting Information.

3.4 | Fruit Composition

Harvest in each experimental plot was done when grapes reached 23.5° Brix. No statistically significant differences in TSS were found on a yearly basis (data not shown), which allowed us to harvest all the treatments at the same time every year and compare the impact of RDI, excluding possible effects associated with harvest time. However, when the cumulative data from the five seasons were analyzed together, slight differences in TSS were found (Table 3). The analysis shows that 35%–100% ETa had a significantly lower average Brix of $\approx 0.5^\circ$ compared to the control and 70% ETa. A significant interaction between the treatment and the season was found for TSS derived from a low TTS on the 70% ETa treatment in the 2019 season (Supporting Information).

Regarding pH, no difference was observed between treatments. Instead, malic acid was highly sensitive to the reductions in irrigation, with 50%–100% ETa and 35%–100% ETa treatments showing reductions of 26% and 37%, respectively. This change in malic acid impacted the TA of the musts, with 50%–100% ETa and 35%–100% ETa treatments having lower TA values than the control treatment (i.e., 6.6% and 5.7% lower, respectively). Significant interactions between treatment and season were found in the malic acid concentration and TA, due to the different

behavior of the 70% ETa treatment in 2019 compared to the rest of the seasons (Supporting Information).

As far as nitrogen content, measured as organic PAN, inorganic ammonia (RAP), and YAN, the most restricted RDI treatments, 50%–100% ETa and 35%–100% ETa, had the highest concentration for both organic and inorganic nitrogen, as well as for YAN, which was 19.7% and 16.3% higher than the control treatment, respectively. On the other hand, 70% ETa, which had a less restrictive but steady RDI, also had a higher nitrogen concentration compared to the control, but in this case, only for PAN and YAN, with no impact on the inorganic RAP nitrogen concentration. Significant interactions of treatment against season were found for PAN and YAN, both associated with a reduced concentration for the 35%–100% ETa treatment in the 2019 season.

The season affected the general composition of the grapes, increasing the °Brix and pH, while reducing the concentration of malic acid and YAN in the warm 2019–2020. This happened even though this season was the earliest harvested. The lowest °Brix, and highest TA and YAN, happened in the 2020–2021 season, the second coldest season with 2020° GDD. The coldest season, 2021–2022, was also characterized by the lowest pH, highest malic acid concentration, and second highest YAN.

Table 4 illustrates the effect of the treatments on traditional phenolic measurements. The results show that optical density (OD 280, 420, 520, and 620 nm), color index (CI), total anthocyanins, and tannins increased significantly with higher RDI intensity. The highest values for all these phenolic measurements were obtained with the 35%–100% ETa treatment. There were no significant differences in the CI (i.e., the sum of OD 420, 520, and 620 nm) between 50% and 100% ETa and 35%–100% ETa treatments; however, both were statistically greater than the control and 70% ETa. Even though no differences in CI were found between the 35%–100% ETa and 50%–100% ETa treatments, increased OD 520 in the 35%–100% ETa treatment was observed, resulting in a more reddish color. Also, a lower optical density at 620 nm in the control treatment compared with the 70% ETa was also found, but without differences in their CI. This means that they had the same color intensity, but they presented variations in their blueish-purple tone.

The effects of RDI on anthocyanin concentration on a fresh-weight basis, measured with the Harbertson–Adams method, followed a similar trend for the 35%–100% ETa treatment, which showed the highest absolute value of all. In the case of 50%–100% ETa no statistically significant differences were found against the control and 70% ETa treatments. Also, no statistical differences were found between 50% and 100% ETa and 35%–100% ETa treatments.

Regarding the total tannin concentration on a fresh-weight basis, both methodologies agree on an increase between 7% and 15% (depending on the methodology) in the two most restricted treatments, 50%–100% ETa and 35%–100% ETa, compared with the control. It is interesting to point out that the highest discrimination capability between the treatments was found with the MCP assay, which also showed more tannins in the 70% ETa treatment compared to the control.

When results were analyzed by season, the coldest ones corresponding to 2020–2021 and 2021–2022 had the highest CI and

TABLE 3 | Effect of regulated deficit irrigation on must basic chemical composition for a drip-irrigated vineyard (cv. Cabernet Sauvignon) over five consecutive seasons.

Treatments	TSS (°brix)	pH	Total acidity (g tartaric acid/L)	Malic acid (g/L)	RAP (mg nitrogen/L)	PAN (mg nitrogen/L)	YAN (mg nitrogen/L)
100% ETa	24.21 ± 0.13 a	3.56 ± 0.02	4.96 ± 0.11 a	1.97 ± 0.08 a	121.7 ± 16.6 b	58.2 ± 3.9 c	161.5 ± 14.7 c
70% ETa	24.33 ± 0.19 a	3.55 ± 0.01	4.85 ± 0.11 ab	1.62 ± 0.08 b	124.7 ± 15.6 b	65.2 ± 3.7 b	172.9 ± 13.8 b
50%–100% ETa	24.07 ± 0.12 ab	3.57 ± 0.02	4.63 ± 0.09 b	1.45 ± 0.06 c	134.7 ± 15.0 a	74.9 ± 3.7 a	193.3 ± 13.3 a
35%–100% ETa	23.75 ± 0.13 b	3.53 ± 0.01	4.68 ± 0.11 b	1.24 ± 0.05 d	132.2 ± 15.5 a	75.0 ± 4.1 a	187.9 ± 13.5 a
P-value treatment	< 0.005	0.239	< 0.005	< 0.001	< 0.001	< 0.001	< 0.001
2017–2018	24.15 ± 0.12 A	3.52 ± 0.01 BC	4.56 ± 0.08 C	1.38 ± 0.04 B	68.0 ± 2.1 C	51.5 ± 1.7 D	119.5 ± 3.2 D
2018–2019	24.16 ± 0.12 A	3.60 ± 0.01 A	4.53 ± 0.08 C	1.36 ± 0.04 B	88.0 ± 2.3 B	60.0 ± 1.7 C	147.0 ± 3.2 C
2019–2020	24.56 ± 0.12 A	3.59 ± 0.01 A	4.87 ± 0.08 B	1.37 ± 0.04 B	68.7 ± 2.1 C	72.9 ± 1.7 B	129.3 ± 3.2 D
2020–2021	23.40 ± 0.12 B	3.56 ± 0.01 AB	5.30 ± 0.08 A	1.82 ± 0.04 A	206.6 ± 2.1 A	97.2 ± 1.7 A	266.1 ± 3.2 A
2021–2022	24.15 ± 0.12 A	3.49 ± 0.01 C	4.96 ± 0.08 BC	1.90 ± 0.04 A	210.3 ± 2.1 A	60.0 ± 1.7 C	232.5 ± 3.2 B
P-value season	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
P-value T x S	< 0.001	0.175	< 0.050	< 0.005	< 0.001	0.153	< 0.005

Note: Different lowercase letters in vertical order indicate significant differences between the treatments, and different uppercase letters in vertical order indicate significant differences between season p -value ≤ 0.05 , Tukey HDS test. RAP (inorganic ammonia).

Abbreviations: PAN, primary amino nitrogen; YAN, yeast assimilable nitrogen.

TABLE 4 | Effect of regulated deficit irrigation on the general phenolic composition of grapes for a drip-irrigated vineyard (cv. Cabernet Sauvignon) over five consecutive seasons.

Treatment	OD 280	OD 420	OD 520	OD 620	Color index	Total anthocyanin adams (mg/kg)	Tannins MCP assay (eq. of g epicatechin/kg)	Tannins adams assay (eq. of g catechin/kg)
100% ETa	87.23 ± 2.9 c	12.14 ± 0.55 c	39.08 ± 1.88 c	3.10 ± 0.23 c	54.31 ± 2.65 b	1183 ± 62 b	3.67 ± 0.16 c	2.22 ± 0.06 b
70% ETa	93.82 ± 2.7 bc	12.83 ± 0.63 bc	40.16 ± 1.83 bc	3.56 ± 0.30 b	55.92 ± 2.61 b	1193 ± 63 b	3.97 ± 0.16 b	2.26 ± 0.06 b
50%-100% ETa	99.87 ± 2.5 ab	13.50 ± 0.61 ab	43.16 ± 1.55 b	3.82 ± 0.31 ab	60.98 ± 2.40 a	1285 ± 49 ab	4.14 ± 0.15 ab	2.36 ± 0.05 a
35%-100% ETa	103.50 ± 3.0 a	14.36 ± 0.51 a	47.12 ± 1.60 a	3.93 ± 0.25 a	65.41 ± 2.30 a	1382 ± 62 a	4.24 ± 0.17 a	2.38 ± 0.06 a
P-value treatment	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
2017-2018	90.00 ± 1.96 B	10.41 ± 0.28 C	34.12 ± 1.13 C	2.05 ± 0.10 D	49.76 ± 1.43 C	945 ± 35 C	3.46 ± 0.08 C	2.05 ± 0.03 D
2018-2019	84.86 ± 1.96 B	12.11 ± 0.28 B	41.70 ± 1.04 B	2.99 ± 0.09 C	56.79 ± 1.32 B	1190 ± 33 B	3.82 ± 0.08 B	2.27 ± 0.03 C
2019-2020	103.27 ± 1.96 A	11.72 ± 0.28 B	37.31 ± 1.08 C	3.24 ± 0.09 C	52.52 ± 1.37 B	1156 ± 36 B	5.16 ± 0.07 A	2.47 ± 0.03 B
2020-2021	91.63 ± 1.96 B	16.04 ± 0.28 A	49.11 ± 1.08 A	5.30 ± 0.10 A	69.81 ± 1.43 A	1561 ± 34 A	3.67 ± 0.08 BC	2.12 ± 0.03 D
2021-2022	110.76 ± 2.03 A	15.76 ± 0.29 A	49.64 ± 1.08 A	4.44 ± 0.10 B	69.87 ± 1.37 A	1450 ± 33 A	3.91 ± 0.08 B	2.62 ± 0.03 A
P-value season	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
P-value T x S	< 0.005	0.311	0.219	0.337	0.545	0.073	0.561	0.262

Note: Different lowercase letters in vertical order indicate significant differences between the treatments, and different uppercase letters in vertical order indicate significant differences between season p -value ≤ 0.05 , Tukey HDS test.

total anthocyanins, while the warmer season (2019–2020) showed a high concentration of tannins, measured by the MCP and Harbertson–Adams assays. Instead, the lowest concentrations of anthocyanins, tannins, and CI were found in the 2017–2018 growing season, which had mild temperatures but the latest harvest of all.

Out of all the traditional phenolic measurements, only OD 280 showed a significant interaction between treatment and season, derived by a reduction in the OD in the 35%–100% treatment in the 2021 season (Supporting Information).

Table 5 shows that water restrictions of 50% of the ETa or higher positively impacted the concentration on a fresh-weight basis of most of the monomeric anthocyanins, particularly the most relevant ones in terms of concentration, such as the malvidin derivatives. On the other hand, reductions of just 70% of ETa did not significantly increase the concentration of any particular anthocyanin or their combined total concentration. Most of the monomeric anthocyanins showed an increased concentration in at least one of the most water-restrictive RDI treatments (i.e., 50%–100% ETa or 35%–100% ETa), thus impacting the total monomeric anthocyanin concentration of these treatments, with increments of 19.6% and 9.3% for the 35%–100% ETa and 50%–100% ETa, respectively. When looking at the increment in anthocyanin concentration, compared to the reduction in yield, it is possible to observe how the 35%–100% ETa treatment had a more considerable gain compared to the 50%–100% ETa treatment, resulting in an additional 10.3% increment in anthocyanin compared to just 4% extra reduction in yield, both based on the control anthocyanin concentration and yield. Only three monomeric anthocyanins did not respond to RDI treatments as the rest of them: peonidin 3-O-glucoside and peonidin 3-O-acetyl-glucoside, showed no differences among treatments, while cyanidin-3-O-glucoside was negatively affected by deficit irrigation. These three anthocyanins are di-hydroxylated, and the anthocyanins that increased with RDI are tri-hydroxylated, except for the peonidin-3-O-coumaroyl-glucoside. RDI had no effect on the total amount of di-hydroxylated anthocyanins accumulated (Table 5), however there was an increase in the total tri-hydroxylated anthocyanins accumulated and also the ratio of tri-hydroxylated/di-hydroxylated (Tri/Di), where the more restrictive the treatment, the higher the ratio.

When looking at the content on a per-berry basis, which takes into account the changes in berry weight and gives a better idea of the concentration mediated by synthesis or degradation, the results indicate a relative reduction of most of the anthocyanins and their combined total concentration in the RDI treatments. This includes the di-hydroxylated peonidin 3-O-glucoside and peonidin 3-O-acetyl-glucoside, which did not show differences on a fresh-weight basis, as well as cyanidin-3-O-glucoside, which exhibited a reduction on a per-berry basis under RDI treatments, consistent with its behavior on a fresh-weight basis. A particular behavior was observed for malvidin-3-O-glucoside and malvidin-3-O-acetyl-glucoside measured on a per-berry basis, where only the 50%–100% ETa treatment was lower than the control, while the 70% ETa and the 35%–100% ETa treatment did not differ from the control treatment. Although these two anthocyanins represented the largest content on a per-berry basis, this lack of differences in content between the control and the 35%–100% ETa did not compensate for the decrease observed in other

anthocyanins. Consequently, the combined effect resulted in a lower total anthocyanin concentration across all the RDI treatments.

On a per-berry basis, the sum of di-hydroxylated anthocyanins did show significant differences; in this case, RDI reduced their concentration. On the other hand, the sum of tri-hydroxylated anthocyanins content per berry was only affected by the 70% ETa treatment, which was lower relative to the control. The ratio of tri-/di-hydroxylated anthocyanins on a per-berry basis was similar to that observed on a fresh-weight basis, increasing as water restriction intensified.

The cold seasons (2020–2021 and 2021–2022) had the highest sum of dihydroxylated, trihydroxylated, and total anthocyanins by HPLC-DAD both as a fresh-weight basis and as a per-berry basis (Table 5), while the warm 2019–2020 had the lowest. With regard to the tri/di ratio, no clear relationship between cold or warm seasons was found, neither as fresh-weight basis nor per-berry basis, although a link with the accumulated GDD at harvest was observed, where low GDD to harvest (2021–2022; 2019–2020; 2020–2021) was related to a low ratio of tri/di, and the opposite to high GDD to harvest (2017–2018; 2018–2019). Regarding the concentration of specific anthocyanins by season, the results show almost the same behavior when expressed on a fresh-weight or a per-berry basis, with some slight differences regarding the magnitude of the differences between treatments. Colder seasons (2020–2021 and 2021–2022) had higher concentration compared to warmer seasons (2019–2020), except for the two coumaroyl anthocyanins measured, malvidin-3-O-coumaroyl-glucoside and peonidin-3-O-coumaroyl-glucoside, which did not show a clear pattern regarding season temperature, either expressed as a fresh-weight or as a per-berry basis. Regarding the treatment $\times$ season interaction, significant effects were observed only for delphinidin-3-O-glucoside and petunidin-3-O-glucoside, both on a fresh-weight and per-berry basis, as well as for cyanidin-3-O-glucoside and the sum of di-hydroxylated anthocyanins on a per-berry basis (see Supporting Information).

Table 6 shows that on a berry fresh-weight basis, the total flavan-3-ols and flavonols were significantly affected by water restriction, whereas phenolic acids exhibited no significant differences among treatments. Catechin concentration was 47.6% (14 mg/kg) lower in the 35%–100% ETa treatment compared with the control, while no significant reductions in epicatechin were found in this trial. On the other hand, the concentration of epicatechin gallate was significantly increased by the RDI treatments, particularly by the most water-restrictive treatments, 50%–100% ETa and 35%–100% ETa. However, this flavan-3-ol had the lowest accumulation, and its contribution is marginal to the total flavan-3-ol concentration, which was 23.9% higher in the control treatment compared with 35%–100% ETa.

When analyzing the concentration of flavan-3-ols on a per-berry basis, similar results to those obtained on a fresh-weight basis were observed, namely a reduction in the concentration mediated by RDI, particularly in the two most water-restrictive treatments. In this case, more pronounced differences between treatments were found, including a lower content of epicatechin per berry in the RDI treatments compared to the control. Additionally, statistically significant differences were observed between the 50%–100% ETa treatment and the control in terms of

TABLE 5 | Effect of regulated deficit irrigation on total monomeric anthocyanin concentration in grapes by HPLC-DAD, and hydroxylation ratio of grapes for a drip-irrigated vineyard (cv. Cabernet Sauvignon) over five consecutive seasons.

Treatment	Units	100% ETa	70% ETa	50%-100% ETa	35%-100% ETa	P-value treatment	2017-2018	2018-2019	2019-2020	2020-2021	2021-2022	P-val season	P-val T x S
Malvidin-3-O-Glc	(mg/kg) (mg/ berry)	337.1 ± 12.3 c 0.3961 ± 0.0161	345.4 ± 14.4 bc 0.3694 ± 0.0187	369.4 ± 11.5 b 0.3557 ± 0.0185 b	403.1 ± 11.9 a 0.3750 ± 0.0165	< 0.001 < 0.050	324.9 ± 8.6 B 0.3037 ± 0.0108 C	348.1 ± 8.6 B 0.4037 ± 0.0108 B	315.5 ± 8.9 B 0.2935 ± 0.0108 C	414.0 ± 8.6 A 0.4497 ± 0.0108 A	418.1 ± 8.6 A 0.4195 ± 0.0108 A	< 0.001 < 0.001	0.059 0.108
Malvidin-3-O-Ac-Glc	(mg/kg) (mg/ berry)	195.2 ± 5.8 c 0.2299 ± 0.0088	203.3 ± 9 bc 0.2142 ± 0.0117	221.5 ± 9 b 0.2012 ± 0.0105 b	248.3 ± 6.2 a 0.2257 ± 0.0092 a	< 0.001 < 0.050	192.8 ± 6.7 B 0.1791 ± 0.0071 C	244.3 ± 6.7 A 0.2498 ± 0.0098	190.8 ± 6.7 B 0.1815 ± 0.0071 C	233.9 ± 6.7 A 0.2535 ± 0.0071 A	223.4 ± 6.7 A 0.2246 ± 0.0071 B	< 0.001 < 0.001	0.111 0.396
Malvidin-3-O-Coum-Glc	(mg/kg) (mg/ berry)	77.2 ± 2.4 c 0.0909 ± 0.0025	84.4 ± 3.5 c 0.0902 ± 0.0049	96.6 ± 4.1 b 0.0943 ± 0.0061	108 ± 2.8 a 0.0990 ± 0.0038	< 0.001 0.130	81.5 ± 3.1 C 0.0751 ± 0.0033 D	105.4 ± 2.9 A 0.1181 ± 0.0034 A	85.8 ± 3.0 BC 0.0798 ± 0.0032	96.1 ± 2.9 AB 0.1037 ± 0.0031 B	88.9 ± 2.9 BC 0.0912 ± 0.0033	< 0.001 < 0.001	0.271 0.162
Delphinidin-3-O-Glc	(mg/kg) (mg/ berry)	64.1 ± 5.6 ab 0.0751 ± 0.0067	57.4 ± 4.5 b 0.0612 ± 0.0050 b	59.7 ± 4.5 ab 0.0579 ± 0.0044 b	66.8 ± 5.4 a 0.0622 ± 0.0053 b	< 0.050 < 0.001	51.1 ± 2.8 C 0.0484 ± 0.0031 B	45.2 ± 2.8 C 0.0524 ± 0.0031 B	44.1 ± 2.8 C 0.0423 ± 0.0031 B	79.1 ± 2.8 B 0.0866 ± 0.0031 A	90.4 ± 2.8 A 0.0907 ± 0.0031 A	< 0.001 < 0.001	< 0.050 < 0.050
Petunidin-3-O-Glc	(mg/kg) (mg/ berry)	49.0 ± 3.3 b 0.0574 ± 0.0040	45.9 ± 2.7 b 0.0490 ± 0.0031 b	49.5 ± 2.9 b 0.0466 ± 0.0028 b	54.4 ± 3.4 a 0.0495 ± 0.0030 b	< 0.050 < 0.050	43.1 ± 1.9 C 0.0406 ± 0.0020	40.6 ± 1.9 C 0.0472 ± 0.0020 B	38.6 ± 1.9 C 0.0361 ± 0.0020 C	58.9 ± 1.9 B 0.0642 ± 0.0020 A	67.3 ± 1.9 A 0.0651 ± 0.0022 A	< 0.001 < 0.001	< 0.050 < 0.050
Peonidin-3-O-Glc	(mg/kg) (mg/ berry)	34.3 ± 1.9 0.0400 ± 0.0021	31.9 ± 1.7 0.0339 ± 0.0019 b	31.9 ± 1.7 0.0307 ± 0.0017	32.0 ± 1.5 0.0296 ± 0.0016 c	0.141 < 0.001	30.3 ± 0.9 B 0.0287 ± 0.0010 B	24.5 ± 0.9 C 0.0286 ± 0.0010 B	27.7 ± 0.9 BC 0.0267 ± 0.0010 B	39.7 ± 1.0 A 0.0432 ± 0.0010 A	40.3 ± 0.9 A 0.0405 ± 0.0010 A	< 0.001 < 0.001	0.396 0.057
Peonidin-3-O-Ac-Glc	(mg/kg) (mg/ berry)	11.4 ± 0.5 0.0135 ± 0.0007	10.6 ± 0.4 0.0115 ± 0.0006 b	11.1 ± 0.4 0.0108 ± 0.0005 b	11.4 ± 0.5 0.0106 ± 0.0005 b	0.232 < 0.001	9.2 ± 0.3 C 0.0087 ± 0.0003 D	9.3 ± 0.3 C 0.0108 ± 0.0003 C	11.1 ± 0.3 B 0.0106 ± 0.0003 C	13.4 ± 0.3 A 0.0151 ± 0.0003 A	12.7 ± 0.3 A 0.0128 ± 0.0003 B	< 0.001 < 0.001	0.903 0.132
Peonidin-3-O-Coum-Glc	(mg/kg) (mg/ berry)	8.4 ± 0.5 b 0.0102 ± 0.0005	8.9 ± 0.4 ab 0.0096 ± 0.0005	9.6 ± 0.4 a 0.0093 ± 0.0005	9.6 ± 0.5 a 0.0091 ± 0.0005 b	< 0.050 < 0.050	7.2 ± 0.2 C 0.0067 ± 0.0003 D	7.2 ± 0.3 C 0.0089 ± 0.0003 C	9.7 ± 0.2 B 0.0092 ± 0.0003 C	11.3 ± 0.3 A 0.0124 ± 0.0003 A	10.2 ± 0.3 B 0.0105 ± 0.0003 B	< 0.001 < 0.001	0.834 0.357
Cyanidin-3-O-Glc	(mg/kg) (mg/ berry)	5.7 ± 0.7 a 0.0066 ± 0.0009	4.4 ± 0.6 b 0.0049 ± 0.0006 b	4.2 ± 0.6 b 0.0039 ± 0.0005	4.0 ± 0.6 b 0.0038 ± 0.0006 c	< 0.001 < 0.001	2.9 ± 0.3 C 0.0031 ± 0.0003 C	0.9 ± 0.2 D 0.0011 ± 0.0003 D	4.8 ± 0.2 B 0.0047 ± 0.0003 B	7.0 ± 0.3 A 0.0077 ± 0.0003 A	7.1 ± 0.3 A 0.0074 ± 0.0003 A	< 0.001 < 0.001	0.104 < 0.050
Total Anthocyanins HPLC	(mg/kg) (mg/ berry)	784.3 ± 29.2 c 0.9412 ± 0.0386	791.8 ± 33.4 c 0.8363 ± 0.0425 b	857.4 ± 27 b 0.8331 ± 0.0460 b	938.1 ± 27.7 a 0.8551 ± 0.0413 b	< 0.001 < 0.050	742.5 ± 22.6 C 0.6769 ± 0.0230 B	826.1 ± 21.8 B 0.9640 ± 0.0238 A	731.3 ± 22.6 C 0.6920 ± 0.0230 B	955.6 ± 21.8 A 1.0344 ± 0.0230 A	959.0 ± 22.0 A 0.9748 ± 0.0239 A	< 0.001 < 0.001	0.055 0.224
Sum of Di-Hydroxylated	(mg/kg) (mg/ berry)	59.7 ± 3.7 0.0693 ± 0.0025	55.9 ± 2.9 0.0598 ± 0.0049 b	56.8 ± 3.0 0.0548 ± 0.0061 c	57.0 ± 2.9 0.0532 ± 0.0038 c	0.408 < 0.001	48.0 ± 1.49 B 0.0463 ± 0.0018 B	41.0 ± 1.49 C 0.0494 ± 0.0017 B	53.3 ± 1.39 B 0.0513 ± 0.0017 B	70.7 ± 1.49 A 0.0781 ± 0.0018 A	69.0 ± 1.50 A 0.0712 ± 0.0017 A	< 0.001 < 0.001	0.330 < 0.050
Sum of Tri-Hydroxylated	(mg/kg) (mg/ berry)	722.6 ± 26.1 c 0.8506 ± 0.0357	741.7 ± 31.2 bc 0.7768 ± 0.0407 b	800.2 ± 25.6 b 0.7788 ± 0.0439	880.7 ± 25.5 a 0.8083 ± 0.0394	< 0.001 < 0.050	700.3 ± 21.5 BC 0.6306 ± 0.0214 B	783.6 ± 20.8 B 0.9139 ± 0.0222 A	677.4 ± 21.5 C 0.6332 ± 0.0206 B	882.1 ± 20.8 A 0.9446 ± 0.0214 A	888.0 ± 20.8 A 0.8957 ± 0.0222 A	< 0.001 < 0.001	0.061 0.402
Tri/Di	(mg/kg) (mg/ berry)	12.5 ± 0.44 d 12.1 ± 0.46 c	13.6 ± 0.64 c 13.3 ± 0.63 bc	14.7 ± 0.76 b 14.4 ± 0.75 ab	16.1 ± 0.53 a 15.8 ± 0.62 a	< 0.001 < 0.001	14.7 ± 0.38 B 14.1 ± 0.42 B	18.3 ± 0.43 A 18.1 ± 0.45 A	12.6 ± 0.38 C 12.5 ± 0.42 BC	12.3 ± 0.39 C 12.2 ± 0.42 C	13.4 ± 0.40 C 12.6 ± 0.42 BC	< 0.001 < 0.001	0.301 0.402

Note: Different lowercase letters in horizontal order indicate significant differences between the treatments, and different uppercase letters in horizontal order indicate significant differences between season p -value ≤ 0.05 , Tukey HDS test.

TABLE 6 | Effect of regulated deficit irrigation on flavan-3-ols, phenolic acids, and flavonol composition of grapes for a drip-irrigated vineyard (cv. Cabernet Sauvignon) over five consecutive seasons.

Treatment	Units	100% ETa	70% ETa	50%–100% ETa	35%–100% ETa	P-value treatment	2017–2018	2018–2019	2019–2020	2020–2021	2021–2022	P-value season	P-value T x S
Catechin	(mg/kg)	43.4 ± 5.9 a	36.2 ± 4.9 ab	35.0 ± 3.7 ab	29.4 ± 2.8 b	< 0.050	41.4 ± 2.7 B	23.0 ± 2.7 C	65.9 ± 2.7 A	21.2 ± 2.7 C	28.5 ± 2.7 C	< 0.001	0.112
	(mg/ berry)	0.0425 ± 0.0037 a	0.0357 ± 0.0038 ab	0.0326 ± 0.0027 bc	0.0265 ± 0.0021 c	< 0.001	0.0370 ± 0.0020 B	0.0273 ± 0.0020 C	0.0554 ± 0.0022 A	0.0233 ± 0.0020 C	0.0286 ± 0.0020 C	< 0.001	0.069
Epicatechin	(mg/kg)	30.2 ± 3.1	27.8 ± 2.6	28.8 ± 2.2	26.1 ± 2.1	0.129	25.5 ± 1.3 B	24.9 ± 1.3 B	47.5 ± 1.3 A	19.4 ± 1.3 C	23.8 ± 2.7 BC	< 0.001	0.437
	(mg/ berry)	0.0337 ± 0.0029 a	0.0292 ± 0.0024 b	0.0274 ± 0.0017 bc	0.0239 ± 0.0018 c	< 0.001	0.0242 ± 0.0015 C	0.0295 ± 0.0015 B	0.0440 ± 0.0015 A	0.0212 ± 0.0014 C	0.0239 ± 0.0014 C	< 0.001	0.191
Epicatechin gallate	(mg/kg)	9.0 ± 0.9 c	10.4 ± 1.2 b	12.1 ± 1.4 a	11.9 ± 1.4 a	< 0.001	7.0 ± 0.4 B	14.1 ± 0.4 A	15.4 ± 0.4 A	2.6 ± 0.4 C	15.1 ± 0.4 A	< 0.001	< 0.001
	(mg/ berry)	0.0106 ± 0.0012	0.0114 ± 0.0014	0.0116 ± 0.0014	0.0114 ± 0.0015	0.059	0.0066 ± 0.0003 C	0.0175 ± 0.0004 A	0.0144 ± 0.0004 B	0.0028 ± 0.0003 D	0.0150 ± 0.0003 B	< 0.001	< 0.050
Total flavan-3-ols	(mg/kg)	84.0 ± 9.5 a	74.5 ± 8.1 ab	74.0 ± 5.6 ab	67.8 ± 5.8 b	< 0.050	73.9 ± 4.1 B	63.7 ± 4.3 B	127.0 ± 4.3 A	43.2 ± 4.1 C	67.4 ± 4.1 B	< 0.001	0.174
	(mg/ berry)	0.0848 ± 0.0061 a	0.0777 ± 0.0076 ab	0.0771 ± 0.0049 bc	0.0618 ± 0.0048 c	< 0.001	0.0666 ± 0.0038 B	0.0734 ± 0.0037 B	0.1143 ± 0.0040 A	0.0473 ± 0.0037 C	0.0675 ± 0.0037 B	< 0.001	0.162
Cultrinic acid	(mg/kg)	10.6 ± 1.4	9.7 ± 1.2	9.9 ± 0.6	10.3 ± 0.8	0.859	6.3 ± 0.9 C	9.4 ± 0.9 BC	14.9 ± 0.9 A	7.6 ± 0.9 C	12.3 ± 0.9 AB	< 0.001	0.423
	(mg/ berry)	0.0105 ± 0.0007	0.0099 ± 0.0007	0.0091 ± 0.0007	0.0094 ± 0.0006	0.320	0.0057 ± 0.0007 C	0.0103 ± 0.0007 AB	0.0127 ± 0.0008 A	0.0083 ± 0.0007 BC	0.0115 ± 0.0008 A	< 0.001	0.131
Caféic acid	(mg/kg)	< LOQ	< LOQ	< LOQ	< LOQ	NA	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	NA	NA
	(mg/ berry)	< LOQ	< LOQ	< LOQ	< LOQ	NA	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	NA	NA
p-Coumaric acid	(mg/kg)	0.82 ± 0.14	0.88 ± 0.16	0.89 ± 0.17	0.81 ± 0.16	0.707	0.78 ± 0.06 C	0.40 ± 0.06 D	1.3 ± 0.06 B	1.7 ± 0.07 A	< LOQ	< 0.001	0.811
	(mg/ berry)	0.0012 ± 0.0001 a	0.0011 ± 0.0001 ab	0.0010 ± 0.0001 ab	0.0009 ± 0.0001 b	< 0.050	0.0007 ± 0.0006 C	0.0004 ± 0.0006 D	0.0013 ± 0.0006 B	0.0016 ± 0.0008 A	< LOQ	< 0.001	0.277
Total phenolic acids	(mg/kg)	11.5 ± 1.4	10.6 ± 1.2	10.8 ± 0.7	11.2 ± 0.9	0.873	7.38 ± 0.94 C	9.80 ± 0.94 BC	16.26 ± 0.94 A	9.47 ± 0.94 BC	12.28 ± 0.94 B	< 0.001	0.425
	(mg/ berry)	0.0138 ± 0.0016 a	0.0105 ± 0.0010 ab	0.0099 ± 0.0006 b	0.0102 ± 0.0007 b	< 0.050	0.0070 ± 0.0010 C	0.0107 ± 0.0010 BC	0.0157 ± 0.0009 A	0.0103 ± 0.00096 BC	0.0117 ± 0.0010 B	< 0.001	0.283
Myricetin-3-O-galactoside	(mg/kg)	7.3 ± 0.4 b	7.7 ± 0.4 b	9.9 ± 0.5 a	10.9 ± 0.5 a	< 0.001	6.6 ± 0.4 B	9.7 ± 0.4 A	9.7 ± 0.4 A	8.8 ± 0.4 A	10.1 ± 0.4 A	< 0.001	0.266
	(mg/ berry)	0.0082 ± 0.0004 b	0.0081 ± 0.0005 b	0.0097 ± 0.0007 a	0.0101 ± 0.0005 a	< 0.050	0.0060 ± 0.0004 C	0.0111 ± 0.0004 A	0.0090 ± 0.0004 B	0.0094 ± 0.0004 AB	0.0097 ± 0.0004 AB	< 0.001	0.183
Myricetin-3-O-glucoside	(mg/kg)	28.3 ± 1.4 b	31.7 ± 1.7 b	40.4 ± 1.9 a	44.0 ± 1.9 a	< 0.001	33.4 ± 1.6 BC	43.1 ± 1.6 A	33.3 ± 1.6 BC	38.7 ± 1.6 AB	31.9 ± 1.6 C	< 0.001	0.086
	(mg/ berry)	0.0334 ± 0.0019 b	0.0340 ± 0.0021 b	0.0400 ± 0.0030 a	0.0401 ± 0.0020 a	< 0.050	0.0305 ± 0.0017 C	0.0500 ± 0.0017 A	0.0311 ± 0.0017 C	0.0417 ± 0.0017 B	0.0318 ± 0.0017 C	< 0.001	0.193
Myricetin	(mg/kg)	1.62 ± 0.21 a	1.02 ± 0.19 b	1.55 ± 0.20 a	1.95 ± 0.21 a	< 0.001	0.48 ± 0.15 C	1.19 ± 0.15 B	2.33 ± 0.15 A	1.53 ± 0.15 B	2.16 ± 0.15 A	< 0.001	< 0.050
	(mg/ berry)	0.0019 ± 0.0002	0.0016 ± 0.0001	0.0016 ± 0.0001	0.0019 ± 0.0001	0.324	0.0011 ± 0.0002 B	0.0014 ± 0.0001 B	0.0022 ± 0.0001 A	0.0016 ± 0.0001 B	0.0026 ± 0.0001 A	< 0.001	< 0.050
Quercetin 3-O-rutinoside	(mg/kg)	3.71 ± 0.24 b	3.68 ± 0.23 b	4.39 ± 0.33 a	4.37 ± 0.30 a	< 0.050	5.01 ± 0.19 A	2.70 ± 0.21 C	3.50 ± 0.19 BC	3.77 ± 0.19 B	5.21 ± 0.19 A	< 0.001	0.274
	(mg/ berry)	0.0046 ± 0.0002 a	0.0039 ± 0.0002 b	0.0040 ± 0.0002 b	0.0037 ± 0.0002 b	< 0.050	0.0046 ± 0.0002 AB	0.0031 ± 0.0002 D	0.0035 ± 0.0002 CD	0.0041 ± 0.0001 BC	0.0049 ± 0.0002 A	< 0.001	0.785
Quercetin 3-O-galactoside	(mg/kg)	39.1 ± 1.9 b	39.6 ± 1.5 b	45.6 ± 1.9 a	45.8 ± 2.1 a	< 0.001	48.5 ± 1.3 A	33.0 ± 1.5 C	42.1 ± 1.4 B	39.9 ± 1.3 B	49.3 ± 0.14 A	< 0.001	0.829
	(mg/ berry)	0.0475 ± 0.0020 a	0.0417 ± 0.0016 b	0.0435 ± 0.0013 b	0.0416 ± 0.0014 b	< 0.050	0.0454 ± 0.0014 AB	0.0388 ± 0.0017 C	0.0411 ± 0.0014 BC	0.0431 ± 0.0014 BC	0.0493 ± 0.0015 A	< 0.001	0.831
Quercetin-3-O-glucoside	(mg/kg)	39.2 ± 4.6 b	40.7 ± 4.8 b	46.7 ± 5.7 a	46.5 ± 5.7 a	< 0.001	43.9 ± 1.6 B	21.8 ± 1.6 D	34.7 ± 1.6 C	29.7 ± 1.6 C	86.2 ± 1.8 A	< 0.001	0.699
	(mg/ berry)	0.0455 ± 0.0054	0.0426 ± 0.0049	0.0435 ± 0.0047	0.0409 ± 0.0048	0.149	0.0411 ± 0.0015 B	0.0252 ± 0.0015 D	0.0329 ± 0.0015 C	0.0320 ± 0.0015 C	0.0844 ± 0.0017 A	< 0.001	0.822
Quercetin	(mg/kg)	1.13 ± 0.05 b	1.06 ± 0.09 b	1.55 ± 0.16 a	1.41 ± 0.14 a	< 0.001	1.70 ± 0.06 A	< LOQ	< LOQ	0.90 ± 0.06 C	1.25 ± 0.05 B	< 0.001	< 0.001
	(mg/ berry)	0.0013 ± 0.00006 ab	0.0011 ± 0.00006 b	0.0014 ± 0.00006 a	0.0012 ± 0.00006 ab	< 0.050	0.0016 ± 0.00005 A	< LOQ	< LOQ	0.0010 ± 0.00005 C	0.0012 ± 0.00006 B	< 0.001	< 0.050
Laricetin hex	(mg/kg)	4.04 ± 0.13 b	4.34 ± 0.15 b	5.17 ± 0.13 a	5.44 ± 0.21 a	< 0.001	5.08 ± 0.16 AB	4.15 ± 0.15 C	5.24 ± 0.15 A	4.62 ± 0.15 BC	4.63 ± 0.15 BC	< 0.001	0.107
	(mg/ berry)	0.0047 ± 0.0015	0.0047 ± 0.0014	0.0051 ± 0.0018	0.0050 ± 0.0014	0.130	0.0047 ± 0.0002	0.0049 ± 0.0002	0.0050 ± 0.0002	0.0050 ± 0.0002	0.0047 ± 0.0002	0.362	0.063
Syringetin-3-O-galactoside	(mg/kg)	6.54 ± 0.37 b	6.99 ± 0.38 b	8.12 ± 0.40 a	8.10 ± 0.33 a	< 0.001	6.85 ± 0.27 C	5.67 ± 0.29 D	7.32 ± 0.27 BC	9.24 ± 0.29 A	8.11 ± 0.27 B	< 0.001	0.488
	(mg/ berry)	0.0078 ± 0.0003	0.0078 ± 0.0003	0.0078 ± 0.0002	0.0074 ± 0.0002	0.519	0.0064 ± 0.0002 C	0.0070 ± 0.0003 BC	0.0069 ± 0.0003 C	0.0101 ± 0.0003 A	0.0081 ± 0.0002 B	< 0.001	0.221
Syringetin-3-O-glucoside	(mg/kg)	7.94 ± 0.42 b	8.58 ± 0.49 b	10.03 ± 0.48 a	10.21 ± 0.42 a	< 0.001	6.68 ± 0.28 D	10.83 ± 0.28 A	10.82 ± 0.28 A	9.67 ± 0.29 B	7.95 ± 0.28 C	< 0.001	0.165
	(mg/ berry)	0.0093 ± 0.0005	0.0094 ± 0.0006	0.0098 ± 0.0007	0.0095 ± 0.0005	0.698	0.0061 ± 0.0004 D	0.0125 ± 0.0004 A	0.0103 ± 0.0004 B	0.0107 ± 0.0004 B	0.0079 ± 0.0004 C	< 0.001	0.082
Kaempferol	(mg/kg)	0.38 ± 0.06 b	0.42 ± 0.02 b	0.61 ± 0.03 a	0.60 ± 0.05 a	< 0.050	0.32 ± 0.03	< LOQ	< LOQ	< LOQ	0.48 ± 0.03	0.302	0.135
	(mg/ berry)	0.0005 ± 0.00004	0.0004 ± 0.00003	0.0005 ± 0.00002	0.0005 ± 0.00003	0.071	0.0005 ± 0.00002	< LOQ	< LOQ	< LOQ	0.0005 ± 0.00002	0.510	< 0.050
Total flavonoids	(mg/kg)	141.7 ± 8.4 b	144.3 ± 6.4 b	173.3 ± 7.1 a	176.6 ± 8.7 a	< 0.001	159.1 ± 5.4 B	130.5 ± 5.4 C	150.7 ± 5.4 BC	147.6 ± 5.4 BC	207.0 ± 5.4 A	< 0.001	0.734
	(mg/ berry)	0.165 ± 0.0080	0.155 ± 0.0065	0.168 ± 0.0060	0.161 ± 0.0068	0.360	0.148 ± 0.0051 B	0.157 ± 0.0055 B	0.144 ± 0.0053 B	0.159 ± 0.0052 B	0.203 ± 0.0054 A	< 0.001	0.718

Note: Different lowercase letters are in horizontal order indicate significant differences between the treatments, and different uppercase letters in horizontal order indicate significant differences between season p -value ≤ 0.05 , Tukey HDS test. < LOQ indicates concentration under the limit of quantification.

total flavan-3-ols concentration, which was not observed on a fresh-weight basis.

There were no significant differences among treatments for caftaric acid, p-coumaric acid, and caffeic acid on a fresh-weight basis; this latter metabolite was, in fact, under the limit of quantification.

On a per-berry basis, p-coumaric acid showed a reduction in content in the 35%–100% ETa treatment. Total phenolic acids were also lower in the two most water-restrictive treatments.

All the different flavonols measured on a fresh-weight basis, as well as the total flavonol concentration on a fresh-weight basis, were consistently higher in the two most water-restrictive treatments, 50%–100% ETa and 35%–100% ETa, compared to control and 70% ETa, with no significant differences between them. The difference between control and 70% ETa was not significant for most flavonol compounds except for myricetin, a minor flavonol in our samples, which was lower for the 70% ETa treatment. This flavonol was the only one not affected by the two most water-restrictive RDI treatments, 50%–100% ETa and 35%–100% ETa. In general, the most abundant flavonols were quercetin, myricetin, and syringetin, particularly their glucosides and galactosides, for which the magnitude of increased concentration on a fresh-weight basis in the different RDI treatments varied between 17% and 61% compared to the control. The highest differences found between RDI treatments against the control were observed on flavonols with low relative concentration, such as kaempferol (+61% for the 50%–100% ETa and 35%–100% ETa), laricitin hex (+47% for 35%–100% ETa), and quercetin (+37% for 50%–100% ETa). The increase in total flavonols between RDI against the control is lower than that, and close to 22%–25% for 50%–100% ETa and 35%–100% ETa treatments, respectively.

Regarding flavonols on a per-berry basis, myricetins tended to follow the same pattern as observed on a fresh-weight basis, with increased concentrations in the two most water-restricted treatments. Quercetins, on the other hand, either lost this effect (i.e., quercetin-3-O-glucoside) or exhibited the opposite behavior, with reduced concentrations in the RDI treatments (i.e., quercetin-3-O-galactoside and quercetin-3-O-rutinoside). For laricitin-hex, siringin-3-O-glucoside and siringin-3-O-glucoside, and kaempferol, no significant differences between treatments were found on a per-berry basis. All these effects resulted in no differences between the treatments when looking at the total flavonols on a per-berry basis.

Regarding the season's effect on the concentration of LMWPs on a fresh-weight basis, the analysis showed that the warm season of 2019–2020 had the highest levels of flavan-3-ols, catechin, epicatechin, and epicatechin gallate, along with an increased concentration of caftaric acid. In contrast, the cooler seasons (2020–2021 and 2021–2022) showed reduced concentrations of flavan-3-ols, catechin, epicatechin, and to some extent epicatechin gallate (just the 2020–2021 season). Even though significant differences in flavonol concentrations were found among seasons, no clear effect was observed due to a particular weather condition, as similar concentrations were recorded in both warm and cool seasons, and their differences might be associated with other variables. When looking at the effects of the season on a per-berry basis, flavan-3-ols, phenolic acids, and

flavonols had the same behavior described as on a fresh-weight basis.

Regarding the treatment $\times$ season interaction, a significant effect was observed only for epicatechin gallate and for free myricetin and quercetin, both on a fresh-weight and per-berry basis. Kaempferol also showed a significant interaction, but only on a per-berry basis (see Supporting Information).

The aroma composition of the samples is summarized in Table 7. The only methoxypyrazine detected was IBMP, and the 70% ETa treatment, with a sustained deficit until harvest, increased its concentration on a fresh-weight basis by 38% compared to the control, but no clear effect was found for the 35%–100% ETa and 50%–100% ETa treatments, which had volatile concentrations between the control and 70% ETa. On a per-berry basis, the 70% ETa treatment also had the highest content of IBMP; however, in this case, significant differences against the two most water-restrictive treatments and the control were found. Within the family of the C6 aldehydes and alcohols, which contribute to the green grassy aroma, hexanal concentration increased with the greater RDI restriction, showing 44%, 35%, and 20% increase against the control in the 35%–100% ETa, 50%–100% ETa, and 70% ETa treatments, respectively. These differences were observed only when expressed on a fresh-weight basis; no significant differences were found on a per-berry basis. On the other hand, trans-2-hexenol was the only C6 alcohol that reduced its concentration with RDI, particularly for the 70% ETa treatment, both on a fresh-weight and per-berry basis.

The terpene aroma family was significantly affected by the RDI strategies, with six of the seven measured molecules being impacted by the treatments on a fresh-weight basis. In these cases, either 50%–100% ETa or 35%–100% ETa had the highest concentration among the treatments. Linalool and nerol, characterized by lavender and orange blossoms aroma, increased their concentration on a fresh-weight basis in the 35%–100% ETa treatment. These differences were particularly higher for nerol, which had a concentration 43% higher than the control. Both cineoles, 1,4 and 1,8-cineole, were more concentrated on a fresh-weight basis in the 50%–100% ETa treatment compared to the control and the 70% ETa treatment (with no differences among control and 70% ETa). A similar result was found for α -terpineol, where the 50%–100% ETa treatment had a concentration on a fresh-weight basis 45% higher than the control and the 70% ETa treatment. No clear effect of the most water-restrictive treatment, 35%–100% ETa, was found for cineoles and α -terpineol, with concentrations between the 50%–100% ETa, the control, and the 70% ETa treatment. Geraniol, on the other hand, exhibited an opposite pattern on a fresh-weight basis, showing higher concentrations under the 70% ETa treatment compared with the two most water-restrictive treatments, while the control had an intermediate concentration against the other treatments.

On a per-berry basis, most of the terpenes did not show an impact associated with irrigation treatment except for linalool, which behaved opposite to that on a fresh weight basis by reducing its content per-berry in the RDI treatments, and for citronellol, which also was reduced by RDI, particularly by the two most water-restrictive treatments. Nerol also showed an effect of RDI on a per-berry basis; however, in this case, increasing water restriction led to higher nerol content, particularly under the two most water-restrictive treatments.

TABLE 7 | Effect of regulated deficit irrigation on aroma composition of grapes for a drip-irrigated vineyard (cv. Cabernet Sauvignon) over five consecutive seasons.

Treatment	Units	100% Efta	70% Efta	50%-100% Efta	35%-100% Efta	P-value treatment	2017-2018	2018-2019	2019-2020	2020-2021	2021-2022	P-value season T x S
3-Isobutyl-2-methoxypyrazine (IBMP)	(ng/kg)	1.49 ± 0.18 b	2.06 ± 0.33 a	1.72 ± 0.31 ab	1.83 ± 0.39 ab	< 0.001	3.64 ± 0.15 A	0.90 ± 0.15 C	2.87 ± 0.15 B	0.49 ± 0.15 C	0.96 ± 0.15 C	< 0.001
	(ng/ berry)	0.0017 ± 0.0002 b	0.0021 ± 0.0003 a	0.0016 ± 0.0002 b	0.0016 ± 0.0003 b	< 0.001	0.0033 ± 0.0001 A	0.0011 ± 0.0001 C	0.0027 ± 0.0001 B	0.0005 ± 0.0001 D	0.0010 ± 0.0001 C	< 0.001
3-Isopropyl-2-methoxypyrazine (IPMP)	(ng/kg)	ND	ND	ND	ND	NA	ND	ND	ND	ND	ND	NA
	(ng/ berry)	ND	ND	ND	ND	NA	ND	ND	ND	ND	ND	NA
Hexanal	(μg/kg)	312 ± 22 c	375 ± 23 b	423 ± 32 ab	452 ± 40 a	< 0.001	271 ± 19 C	271 ± 18 C	404 ± 18 B	529 ± 18 A	479 ± 18 A	< 0.001
	(μg/ berry)	0.3664 ± 0.0261	0.3988 ± 0.0247	0.4098 ± 0.0327	0.4211 ± 0.0406	0.109	0.2523 ± 0.0203 D	0.3198 ± 0.0188 C	0.3754 ± 0.0188 C	0.5701 ± 0.0188 A	0.4775 ± 0.0188 B	< 0.001
Trans-2-hexenol	(μg/kg)	191 ± 34 a	140 ± 25 b	192 ± 31 a	206 ± 33 a	< 0.050	91 ± 15 CD	110 ± 15 C	275 ± 15 B	374 ± 15 A	55 ± 15 D	< 0.001
	(μg/ berry)	0.2132 ± 0.0395 a	0.1505 ± 0.0279 b	0.1901 ± 0.0325 ab	0.1953 ± 0.0336 ab	< 0.050	0.0837 ± 0.0165 CD	0.1347 ± 0.0165 C	0.2567 ± 0.0165 B	0.4061 ± 0.0165 A	0.0551 ± 0.0165 D	< 0.001
cis-3-Hexen-1-ol	(μg/kg)	38.2 ± 5.3	34.4 ± 4.5	42.4 ± 7.2	44.4 ± 7.3	0.166	51.6 ± 3.7 B	41.0 ± 3.7 B	75.5 ± 4.0 A	8.9 ± 3.7 C	22 ± 3.7 C	< 0.001
	(μg/ berry)	0.0420 ± 0.0057	0.0363 ± 0.0048	0.0378 ± 0.00056	0.0375 ± 0.0053	0.470	0.0484 ± 0.0032 B	0.0482 ± 0.0033 B	0.0635 ± 0.0038 A	0.0097 ± 0.0032 C	0.0223 ± 0.0033 C	< 0.001
Trans-3-hexen-1-ol	(μg/kg)	39.8 ± 4.0	38.7 ± 2.8	41.0 ± 3.1	42.8 ± 2.9	0.601	50.5 ± 2.3 A	39.5 ± 2.5 B	51.6 ± 2.3 A	39.2 ± 2.3 B	22.1 ± 2.3 C	< 0.001
	(μg/ berry)	0.0424 ± 0.0038	0.0413 ± 0.0030	0.0393 ± 0.0027	0.0392 ± 0.0027	0.679	0.0447 ± 0.0023 A	0.0439 ± 0.0025 A	0.0498 ± 0.0022 A	0.0422 ± 0.0022 A	0.0221 ± 0.0022 B	< 0.001
1,4-Cineole	(μg/kg)	2.09 ± 0.22 b	2.17 ± 0.18 b	2.71 ± 0.27 a	2.54 ± 0.23 ab	< 0.050	3.61 ± 0.16 A	1.40 ± 0.16 C	2.32 ± 0.16 B	1.50 ± 0.16 C	3.05 ± 0.16 A	< 0.001
	(μg/ berry)	0.0024 ± 0.0002	0.0023 ± 0.0001	0.0025 ± 0.0002	0.0021 ± 0.0002	0.231	0.0031 ± 0.0001 A	0.0016 ± 0.0001 C	0.0022 ± 0.0001 B	0.0016 ± 0.0001 C	0.0030 ± 0.0001 A	< 0.001
1,8-Cineole	(μg/kg)	2.27 ± 0.39 b	2.39 ± 0.15 b	3.11 ± 0.43 a	2.61 ± 0.28 ab	< 0.050	3.31 ± 0.21 B	1.38 ± 0.21 C	2.74 ± 0.21 B	4.69 ± 0.23 A	0.86 ± 0.20 C	< 0.001
	(μg/ berry)	0.0026 ± 0.0004	0.0025 ± 0.0003	0.0029 ± 0.0004	0.0024 ± 0.0003	0.336	0.0032 ± 0.0002 B	0.0016 ± 0.0002 C	0.0025 ± 0.0002 B	0.0048 ± 0.0002 A	0.0009 ± 0.0002 C	< 0.001
Linalool	(μg/kg)	14.6 ± 2.0 b	14.6 ± 1.8 b	14.8 ± 1.9 ab	15.1 ± 1.9 a	< 0.001	1.8 ± 0.1 D	8.6 ± 0.1 C	21.4 ± 0.1 A	20.3 ± 0.1 B	21.6 ± 0.1 A	< 0.001
	(μg/ berry)	0.0168 ± 0.0022 a	0.0155 ± 0.0019 b	0.0143 ± 0.0017 c	0.0140 ± 0.0017 c	< 0.001	0.0017 ± 0.0003 D	0.0101 ± 0.0003 C	0.0204 ± 0.0003 B	0.0218 ± 0.0003 A	0.0217 ± 0.0003 A	< 0.001
α-Terpinol	(μg/kg)	76 ± 18 c	77 ± 15 bc	110 ± 40 a	103 ± 26 ab	< 0.050	257 ± 17 A	83 ± 17 B	59 ± 17 BC	26 ± 17 D	32 ± 17 CD	< 0.001
	(μg/ berry)	0.0804 ± 0.0157	0.0796 ± 0.0144	0.0826 ± 0.0134	0.0814 ± 0.0157	0.989	0.1998 ± 0.0067 A	0.0903 ± 0.0059 B	0.0546 ± 0.0057 C	0.0280 ± 0.0057 D	0.0321 ± 0.0057 CD	< 0.001
Citronellol	(μg/kg)	9.4 ± 1.5	9.2 ± 1.5	9.3 ± 1.6	10.7 ± 1.8	0.195	15.3 ± 0.4 A	10.6 ± 0.4 B	3.0 ± 0.4 C	< LOQ	< LOQ	< 0.001
	(μg/ berry)	0.0109 ± 0.0017 a	0.0097 ± 0.0016 ab	0.0090 ± 0.0014 b	0.0095 ± 0.0015 b	< 0.050	0.0142 ± 0.0004 A	0.0123 ± 0.0004 B	0.0028 ± 0.0004 C	< LOQ	< LOQ	< 0.001
Nerol	(μg/kg)	145 ± 15 b	163 ± 15 ab	189 ± 27 ab	208 ± 34 a	< 0.001	128 ± 17 B	93 ± 19 B	106 ± 17 B	308 ± 21 A	245 ± 19 A	< 0.001
	(μg/ berry)	0.1469 ± 0.0208 b	0.1639 ± 0.0195 ab	0.1835 ± 0.0274 a	0.1957 ± 0.0344 a	< 0.050	0.1235 ± 0.0189 B	0.0967 ± 0.0189 B	0.1001 ± 0.0189 B	0.2878 ± 0.0233 A	0.2543 ± 0.0189 A	< 0.001
Geraniol	(μg/kg)	83 ± 11 ab	96 ± 12 a	80 ± 7 b	76 ± 9 b	< 0.050	121 ± 8 A	87 ± 9 B	59 ± 8 C	< LOQ	67 ± 8 BC	< 0.001
	(μg/ berry)	0.0957 ± 0.0125	0.0999 ± 0.0129	0.0838 ± 0.0123	0.0721 ± 0.0093	0.064	0.1265 ± 0.0094 A	0.1022 ± 0.0102 A	0.0557 ± 0.0094 B	< LOQ	0.0672 ± 0.0094 B	< 0.001
β-Damascenone	(μg/kg)	101 ± 11 b	117 ± 14 ab	140 ± 20 a	105 ± 9 ab	< 0.050	151 ± 13 A	132 ± 13 AB	75 ± 13 B	139 ± 13 A	62 ± 13 B	< 0.001
	(μg/ berry)	0.1176 ± 0.0125	0.1222 ± 0.0139	0.1160 ± 0.0159	0.1010 ± 0.0111	0.249	0.1520 ± 0.0101 AB	0.1223 ± 0.0109 B	0.0708 ± 0.0101 C	0.1645 ± 0.0105 A	0.0615 ± 0.0101 C	< 0.001
β-Ionone	(μg/kg)	11.7 ± 4.3	17.8 ± 6.1	15.8 ± 4.7	14.1 ± 3.6	0.444	1.83 ± 2.9 C	1.6 ± 2.9 C	14.9 ± 2.9 B	48.5 ± 2.9 A	7.5 ± 2.9 BC	< 0.001
	(μg/ berry)	0.0093 ± 0.0017	0.0091 ± 0.0015	0.0082 ± 0.0013	0.0085 ± 0.0013	0.406	0.0017 ± 0.0003 D	0.0018 ± 0.0005 D	0.0141 ± 0.0005 B	0.0187 ± 0.0011 A	0.0075 ± 0.0005 C	< 0.001
Benzyl Alcohol	(μg/kg)	291 ± 43	313 ± 51	331 ± 60	338 ± 55	0.793	128 ± 20 C	158 ± 20 C	767 ± 20 A	305 ± 20 B	247 ± 20 B	< 0.001
	(μg/ berry)	0.3604 ± 0.0660	0.3256 ± 0.0501	0.3116 ± 0.0530	0.2984 ± 0.0447	0.218	0.1172 ± 0.0238 D	0.1857 ± 0.0238 CD	0.7365 ± 0.0238 A	0.3303 ± 0.0238 B	0.2503 ± 0.0238 BC	< 0.001
Benzaldehyde	(μg/kg)	236 ± 44	241 ± 43	258 ± 51	281 ± 55	0.149	439 ± 25 A	412 ± 23 A	369 ± 23 A	32 ± 23 B	21 ± 23 B	< 0.001
	(μg/ berry)	0.2939 ± 0.0549	0.2459 ± 0.0445	0.2660 ± 0.0538	0.2648 ± 0.0510	0.412	0.4488 ± 0.0254 AB	0.4836 ± 0.0254 A	0.3494 ± 0.0254 B	0.0352 ± 0.0254 C	0.0211 ± 0.0255 C	< 0.001

Note: Different lowercase letters in horizontal order indicate significant differences between the treatments, and different uppercase letters in horizontal order indicate significant differences between season p-value ≤ 0.05, Tukey HDS test. < LOQ indicates concentration under the limit of quantification, and ND indicates concentration under the limit of detection.

β -damascenone was the only norisoprenoid affected by RDI, and only when measured on a fresh-weight basis, and just like α -terpineol, the 50%–100% ETa treatment had a significantly higher concentration than the control, but in this case, it was not different from the 70% ETa and 35%–100% ETa treatments.

Regarding the benzene derivatives, benzyl alcohol, and benzaldehyde, RDI did not significantly affect their concentrations, either on a fresh-weight or per-berry basis.

When the results were analyzed after grouping the samples by season, significant differences were found. Differences were practically the same when expressed on a fresh-weight or per-berry basis. Only a few effects over metabolites founded were consistent with warm against cool seasons, for example, cis-3-hexen-1-ol and benzaldehyde tended to have lower concentrations on both as a fresh-weight and as a per-berry basis in cooler seasons (2020–2021; 2021–2022) and higher in warmer seasons (2019–2020), while nerol, also as both a fresh-weight and as a per-berry basis, showed lower concentrations in warmer seasons and high concentrations in cold seasons.

Other aromas seem to be affected by the GDD accumulated at harvest, where high GDD to harvest together with a late harvest, such as 2017–2018, resulted in higher concentration of terpenes, such as α -terpineol, citronellol, and geraniol, while cold and low GDD accumulated at harvest (2019–2020; 2020–2021; 2021–2022) resulted in low concentration of these aromas, both as a fresh-weight and as a per-berry basis.

Regarding the interaction between treatment and season for aroma composition, only a few aroma compounds showed significant treatment $\times$ season effects. These interactions appear to be minor and largely attributable to the absence of treatment differences in certain seasons or to isolated seasonal variations in treatment responses (see Supporting Information).

4 | Discussion

The water restriction levels obtained by the different RDI treatments studied were enough to produce significant differences in vine water status and fruit composition. Most of the differences among treatments with regard to plant water status were observed in the last 4 weeks leading to *veraison*. It is worth noting that given the water restrictions employed in this experiment are based on an incomplete replenishment of the water consumed by the plants through evapotranspiration, and not on maintaining a particular plant water status, as attempted in other experiments, the vine water status in this trial was variable over time.

Regarding seasonal climate, water use, and water productivity, the results show that colder seasons were characterized by lower ETo and ETa. During these periods, RDI treatments showed greater differences in water use compared to the control and exhibited the most significant improvements in water productivity, particularly in treatments applying 70% ETa and 35%–100% ETa.

In contrast, warmer seasons such as 2019–2020 had higher ETo and ETa, and the RDI treatments resulted in smaller gains in both water savings and water productivity.

The reduction in Ψ_x md as the season progresses is a typical behavior observed across all the seasons, particularly in RDI treatments, as noted in other studies [15, 68, 69]. This behavior,

driven by increased environmental water demand as the season advances, is crucial for the RDI strategy, since it helps the RDI treatments to achieve a significantly different Ψ_x md from the control vines during phenological stages when important processes of fruit development occur, such as cell division and expansion, and phenolic compound synthesis. If the ripening process occurred at a different time, when the environmental demand is not the highest, as in other fruits, it would be challenging to have differences in Ψ_x md during the phenological stages where these changes in chemical composition are modulated.

The impact of the RDI treatments on yield components and vine balance was clear in all seasons studied. Like most of the research previously published, we found that the reduction in yield is associated with the level of water restriction of the applied RDI treatment [2, 7, 70]. However, the period during which water restrictions occurred is the most relevant aspect of this relationship. In this study, the 50%–100% ETa and 35%–100% ETa treatments only had a reduction in irrigation in the period between BS to *veraison*, after that, even if the irrigation was increased to fully replenish ETa, the impact of the RDI in that brief period was substantial enough to impact the final yield and several other traits. This supports previous evidence suggesting that the water status in the period between BS to *veraison* is more relevant than in the period *veraison* to harvest in terms of setting the final yield and berry weight [19, 20]. Compared to the 70% ETa treatment, which maintained a constant water restriction, the reduction in water applied throughout the entire season was nearly the same as for the 50%–100% ETa treatment, 26.7% against 28.2%, but the impact on yield was not as severe, demonstrating that the intensity in that period is more relevant than the total amount of applied water during the season. This might also be relevant to the way in which the water stress in these trials is represented compared to other studies, since another popular approach is used to demonstrate treatment differences by accumulated water stress integral, which does not consider the period where the stress was stronger.

In the case of pruning weight, even if it was affected by the intensity of the water restriction, it behaved more in line with the long-term water reduction and the constant impact of water deficiency of the 70% ETa treatment, having a more considerable impact on the final pruning weight. In fact, the proportion of pruning weight reduction of this treatment compared to the yield reduction was considerably higher, leading to an increase in the Ravaz index compared to the other RDI treatments, in which the reduction of yield against pruning weight was proportional to the control.

As indicated before, the combination between the reduction in berry weight and the number of berries per cluster caused the final reduction in cluster weight in the RDI treatments. Even if berry weight was almost linearly reduced as irrigation was diminished, a lack of difference in berry number between the two most water-restrictive treatments, 50%–100% ETa and 35%–100% ETa, resulted in no differences in cluster weight and final yield between these two treatments.

When examining the effects of RDI treatments on grape composition on a yearly basis, the differences in some variables, such as sugar accumulation, are small enough to be hidden under the experimental error and natural variability, unless longer term or

large-scale experiments, such as those of this trial, are performed. In this study, RDI reduced the accumulation of sugars, particularly for the 35%–100% ETa, the most water-restrictive treatment, possibly as a result of reduced photosynthesis, something that was indirectly evaluated as stomatal conductance, which is not included in this article, since it behaved similarly to Ψ_x md, giving no extra information for the purpose of this study. This trait, Ψ_x md, was highly reduced by limited irrigation. This potential photosynthetic limitation will impact the maturation process by not creating new sugars that can be sent and accumulated in the fruit. Similar results showing a reduction of sugar accumulation due to RDI treatments were found by [18, 71, 72].

Another important observation of the study is that the acidity reduction did not perfectly align with the sugar accumulation, in particular for the 35%–100% ETa treatment. Normally, high sugar accumulation will be accompanied by low acidity. In this case, the RDI had a negative impact on the acid concentration, probably explained by the higher rates of malic acid respiration with increasing temperatures [73–76]. Since RDI treatments tend to reduce canopy growth and increase the light exposure of the clusters, berries will increase their temperature, leading to higher rates of malic acid respiration that will finally reduce the TA of the grape must.

With regard to the impact of RDI on the concentration of phenolics, color, and other indices on a fresh-weight basis, which is the most appropriate metric for assessing the potential effects on final wine composition, the results are well aligned with other publications [6, 8, 40, 77]. The total concentration on a fresh-weight basis of anthocyanins increases with RDI, although considerable irrigation reductions must be implemented to generate a significant impact. For instance, reductions of 70% of ETa were not enough to significantly modify the final concentration of most individual or total anthocyanins. These changes are aligned with the color intensity of the grape homogenates and wines (data not shown), which were higher in the 50%–100% ETa and 35%–100% ETa treatments compared to the control and 70% ETa treatment.

Possibly, a severe water reduction before the lag phase during cell division creates a significant difference not only in the total anthocyanin concentration but also in the total tannin concentration of grapes. In fact, almost the same changes observed in anthocyanin concentration were found for the total tannin concentration on a fresh-weight basis. The MCP assay showed differences between the control, 70% ETa, and 35%–100% ETa treatments, while the Harbertson–Addams assay found differences between the groups 50%–100% ETa/35%–100% ETa treatments and control/70% ETa treatments, meaning that the MCP assay has a better discrimination capacity.

When comparing the concentration on a per-berry basis, the results show that in reality, most of the anthocyanins decreased or maintained their concentration when RDI was imposed, mediated either by a reduction in synthesis or by an increased degradation, which in the end resulted in a reduced total anthocyanin concentration mediated by RDI. The only parameter on the anthocyanin profile on a per-berry basis that maintained the same effect as a fresh-weight basis was the tri/di ratio, which increased with the RDI intensity. This ratio is relevant to wine production since a higher ratio will imply a larger proportion of more stable tri-hydroxylated anthocyanins.

The increments observed in the concentration of monomeric anthocyanins on a fresh-weight basis and the reduction on a per-berry basis possibly reflect both a concentration phenomenon mediated by the reduction in berry size and changes in metabolism associated with the degradation of anthocyanins and the modulation of the polyphenolic pathway [6, 21–23, 78]. The concentration effect can be confirmed by relating the lack of differences when comparing the treatments on a per-berry basis against the increased concentration observed when comparing the RDI treatments on a fresh-weight basis. These differences can only be attributed to a concentration effect mediated by reductions in berry size. Also, the degradation and/or metabolism effect of RDI can be discussed by looking at the per-berry data, where RDI reduced most anthocyanins; this can only be achieved by a reduced synthesis or an increased degradation, potentially mediated by increased temperature [79, 80]. Also, the impact of RDI treatments on the anthocyanin profile, particularly the Tri/Di ratio, could be related to a switch in the pathway where metabolites either follow the F3'H path (flavonoid 3'-hydroxylase) or the F3'5'H (flavonoid 3',5'-hydroxylase) path as described by [81], thus suggesting that irrigation may promote the synthesis path of the F3'H, increasing the relative proportion of di-hydroxylated cyanidin-derived anthocyanins, such as cyanidin-3-O-glucoside, and the 3'-O-methylated derivatives derived from cyanidin, such as peonidin-3-O-glucoside and peonidin-3-O-acetyl-glucoside, pigments which are more in the blue spectrum [78, 82], something that also our data show in terms of anthocyanin composition as well as color spectrum. On the other hand, a deficit irrigation may upregulate the F3'5'H path [77, 81], increasing the synthesis of tri-hydroxylated delphinidin-derived anthocyanins like delphinidin-3-O-glucoside, petunidin-3-O-glucoside, malvidin-3-O-glucoside, malvidin-3-O-acetyl-glucoside, and malvidin-3-O-coumaroyl-glucoside; these anthocyanins, in terms of relative concentration, are the most relevant, considerably impacting the total concentration of anthocyanins [82], something that also was found in this study.

The results obtained in this work are relevant in terms of wine color stability, given that a higher concentration of tri-hydroxylated anthocyanins in grapes may contribute to having a more stable color in wine, resulting from their ability to interact with other molecules and their increased resistance to pH changes and oxidation [83–85]. RDI promotes a higher relative concentration of tri-hydroxylated anthocyanins, which, along with the other changes in color-related phenolics mediated by RDI, could possibly allow the production of wines with a higher overall color [86]. Our data also found that neither warm nor cold seasons increase this ratio, but seasons with extended periods of ripening before harvest do (GDD to harvest).

The findings of this study related to flavonols, which increased under RDI, support the idea of RDI upregulating several genes including flavonol synthase (FLS) as previously described by [81] and by indirect effects mediated by RDI such as increased light exposure associated with less vigorous canopies [87]. Our data show how the RDI increases the concentration of nearly all the measured flavonols on a fresh-weight basis, both free and glycosylated, with one exception, that of free myricetin. However, when the data are analyzed on a per-berry basis, thus excluding the concentration effect caused by smaller berry size under RDI treatments, the results suggest that the potential increase in flavonol synthesis, likely mediated by FLS upregulation, is only

partial. This increase does not extend to all flavonols nor to the total flavonol concentration. Specifically, only myricetin-3-O-glucoside and myricetin-3-O-galactoside maintained elevated concentrations per-berry, indicating a possible synthesis enhancement during berry development. In contrast, quercetin-3-O-glucoside showed no significant increase under RDI treatments, and quercetin-3-O-galactoside even exhibited a reduction in concentration.

In the case of flavan-3-ols, the observed reduction in concentration on a fresh-weight basis under RDI treatments indicates that the decrease cannot be attributed to berry size-related concentration effects. When analyzed on a per-berry basis, the differences between the control and RDI treatments became even more pronounced, confirming a genuine reduction in flavan-3-ol content due to RDI. This reduction was particularly evident in the most abundant compounds, catechin and epicatechin. Since flavan-3-ols are distributed in both the skin and seeds, and since our measurements did not differentiate between these tissues, it remains unclear whether the observed decrease is due to reduced accumulation in the skin, the seeds, both, or a shift in the seed-to-pulp ratio among treatments. The same discussion applies to phenolic acids which showed reductions mediated by RDI.

When looking at the results of aroma composition expressed on a fresh-weight basis, it is clear that for the majority of volatiles, a deficit irrigation will increase their concentration, in some cases almost linearly as for hexanal, linalool, and nerol, with the highest concentration observed at the most water-restrictive RDI treatments (i.e., 50%–100% ETa and 35%–100% ETa). Cineoles, α -terpineol, and β -damascenone were more concentrated in the 50%–100% ETa treatment, suggesting that water restrictions should be applied during berry development stages between berry set and *veraison*. On the other hand, molecules like IBMP increase when the deficit irrigation is sustained during the entire berry development period, as in the 70% ETa treatment. The increase in IBMP in this treatment was evident both on a fresh-weight and per-berry basis. However, under the two most water-restrictive treatments, IBMP concentrations decreased on a per-berry basis, suggesting that more severe water stress may actually reduce IBMP levels. Notably, the reduction in berry size under two most water-restrictive treatments led to a concentration effect on a fresh-weight basis that masked the decrease in per-berry IBMP content, indicating that the observed reduction on a per-berry basis is likely due to degradation. Other aromas where the concentration phenomenon mediated by reductions in berry weight masked a decrease in concentration on a per-berry basis were linalool and citronellol, which were reduced by RDI treatments, particularly by the two most water-restrictive treatments. Other aromas such as cineoles, α -terpineol, nerol, and β -damascenone did not show differences on a per-berry basis, meaning that in this case the concentration found as a fresh-weight basis on the RDI treatments is mainly driven by a reduction in berry size.

Other work conducted elsewhere has shown that defoliation treatments have also resulted in higher concentrations of some volatiles like linalool [88]. Nevertheless, summarizing our results, it is important to recognize that independently of the water restriction level, or the period of irrigation restriction, RDI will most likely increase the concentration of aromas on the grapes, either positives such as α -terpineol and β -damascenone or undesirables such as IBMP.

The period during which the water restriction was applied, as well as the level of difference in plant water status, is key to effectively compare the outcomes of this and other trials. Also, since water restrictions are based on ETa replenishment, the different variables associated with the ET demand such as temperature, relative humidity, radiation, and wind speed [89] must be independently considered, as they impact vine behavior in different ways. For instance, for the same ET demand, the impact will be different, depending on whether originated by high wind and low RH or by elevated temperature and high radiation. This is an important consideration to keep in mind when comparing results between regions, since the same RDI will have a different impact on the vine depending on the contribution of each of these variables on the ET. Like so, an additional idea to ponder is that the concept of stress measured as stem water potential depends not only on soil water availability but also on the environment demand, which is based on the variables already listed.

Despite several positive impacts of RDI on grape composition, a clear reduction in yield mediated by RDI must be considered. In this trial, to achieve most of the positive effects, RDI needs to reach at least 50% of the ETa, particularly from pea size to *veraison*. Treatments like 70% ETa, with a consistent low water restriction, did not significantly enhance grape composition compared to the control. However, it results in a considerable yield reduction.

5 | Conclusions

RDI is a technique that, besides contributing to saving water, can have significant impacts on vine productivity and composition traits. The changes observed in grape chemical composition are mainly associated with a concentration phenomenon derived from a reduced berry size, and to changes in the metabolism of grapes. TA, TSS, and phenolics most likely respond to changes in metabolism, including respiration, degradation, and changes in the metabolic route of synthesis. The increased concentration of anthocyanins under RDI treatments is primarily driven by the reduction in berry weight, rather than by enhanced biosynthesis. On a per-berry basis, RDI leads to a decrease in anthocyanin concentration, indicating that the observed increase on a fresh-weight basis is largely a concentration effect. Similarly, flavonol concentration on a berry fresh-weight basis is mainly influenced by berry size reduction; however, specific compounds such as myricetin-3-O-glucoside and myricetin-3-O-galactoside showed increased concentrations on a per-berry basis, suggesting genuine upregulation of synthesis or reduced degradation in those cases. Flavan-3-ols, on the other hand, actually get reduced on the fruit, both as a fresh-weight and a per-berry basis, with no effects associated with berry weight. With regard to aromas, the increased concentration found on a fresh-weight basis is mainly driven by reduction in berry size, since most aromas do not show significant differences mediated by RDI on a per-berry basis, and some of them even show a reduction on a per-berry basis, i.e., cineoles, α -terpineol, nerol, and β -damascenone, that was masked by the concentration effect mediated by berry size. Independently of the mechanisms explaining the changes in concentration on a fresh-weight basis that were observed, most of them are positive with respect to wine quality, with increasing concentrations of positive metabolites and characteristics such as

lower TSS, higher anthocyanins with a higher proportion of tri-hydroxylated, increased YAN, and increased flavonol concentration. Regarding the effect of RDI treatments on aroma composition, the overall effect of RDI is less clearly beneficial, since most of the aromas were more concentrated under RDI treatments, including positive and potentially negative aromas.

Although deficit irrigation contributes to positive changes in grape composition, the specific protocol and intensity of RDI are crucial. Slight restrictions, such as 70% of ETa, even if constant and maintained until harvest, will not generate a substantially positive impact and will only reduce yield, which is the main adverse effect of RDI. To achieve most of the positive impacts, RDI needs to reach at least 50% of the ETa, particularly during pea size to veraison.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data can be shared upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. (*Supporting Information*)

Annex I: Reference standards for chromatography analysis. Annex II: Statistically significant interactions between treatment and season.