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# Effects of Zeowine and compost on leaf functionality and berry composition in Sangiovese grapevines

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## Abstract

Meteorological extremes such as heatwaves and water limitations during the ripening season could negatively impact vine ecophysiology and berry metabolism resulting in lower yield per vine. This project aimed to compare two different soil managements during two growing-production seasons (2021 and 2022) with respect to control without any treatment (control). The two managements were: Zeowine (30 t/ha; a soil conditioner made with clinoptilolite and compost proceeding of industrial wine-waste) and compost (20 t/ha). The trial was organized at Col d'Orcia Estate (Montalcino, Tuscan wine region, Italy). The purpose was twofold: (1) to evaluate the effects of Zeowine treatments on leaf gas exchanges, midday stem water potential, chlorophyll fluorescence and leaf temperature (ecophysiology); and (2) to determine any repercussions on the quality of the grapes (technological and phenolics analyses). The parameters plant yield, yeast assimilable nitrogen, fractionation of anthocyanins (cyanidin, delphinidin, malvidin, peonidin and petunidin), caffeic acid, coumaric acid, gallic acid, ferulic acid, kaempferol and quercetin were also analysed. Zeowine showed higher photosynthesis, less negative midday water potential and lower leaf temperature. Essentially, no significant difference was found between the compost and the control. Furthermore, Zeowine grapevines showed higher anthocyanin accumulation and less quercetin content. In general, compost applied together with zeolite could alleviate the adverse effects of water stress and improve plant growth, yield and quality. The control management strategy proved to be the least beneficial for the well-being of the plant and the final quality of the product, confirming the need for amendments in critical years.

## Introduction

According to the Intergovernmental Panel on Climate Change, during the 20th century the Earth's surface warmed  $\sim 0.75^\circ\text{C}$  (Raymond *et al.*, 2020). The increase of  $\text{CO}_2$  as anthropic-origin carbon dioxide emissions is considered the primary starting point of the detected warming (Vaz *et al.*, 2022). Furthermore, the atmospheric  $\text{CO}_2$  concentration is expected to continue to increase, resulting in higher Earth surface temperatures over the 21st century (Gutiérrez-Gamboa *et al.*, 2021). The persistent climate shift is having an overwhelming impact on global viticulture. In Europe, from 1950 to 2010, the temperatures during the growing season have increased by  $1.7^\circ\text{C}$  (Erlat and Türkeş, 2012). Moreover, by 2050, it is predicted that the temperature range for numerous wine regions will increase by  $0.45^\circ\text{C}$  per decade to a total of  $2.05^\circ\text{C}$  (Jones *et al.*, 2005).

Warming trends necessarily alter atmospheric composition, the balance of organic matter and the soil water balance (Smith *et al.*, 2008). The quantity of soil organic matter is affected by the inputs of waste production and added soluble organic material with decomposition and leaching as output. Climate alteration influences the input and output too through effects on net primary production by changing decomposition and leaching rates (Tóth *et al.*, 2007).

Ecological drought is defined as 'an episodic deficit in water availability that drives ecosystems beyond thresholds of vulnerability, impacts ecosystem services, and triggers feedback in natural and/or human systems' (Crausbay *et al.*, 2017). This deficit is driven by climate variability processes such as the occurrence of periods with below average precipitation amounts or with increased atmospheric evaporative demand (Christensen *et al.*, 2004; Kumar, 2012). Drought can trigger an alteration in hydrological processes (e.g. percolation, soil infiltration, rainfall interception and runoff) and influence the availability of surface and subsurface water resources (Brown *et al.*, 2005; Haj-Amor and Bouri, 2020). On one hand, climate change is expected to increase the frequency, intensity and duration of heat waves, and on the other hand, it is expected to change the normal distribution of rainfall throughout the year which will aggravate heat-related abiotic stress and drought (Gasparrini *et al.*, 2017; Heo *et al.*, 2019).



Heat waves were defined as ‘periods of at least three consecutive days when the maximum and the minimum temperature were simultaneously greater than their respective 95th percentile in Mediterranean environments: 30.0° and 17.3°C, respectively’ (Rey *et al.*, 2007).

Environmental stress factors (high temperature and drought) cause changes in plant metabolism and induce the generation of reactive oxygen species (ROS) (Das and Roychoudhury, 2014). Cellular redox homeostasis is interrupted when ROS generation surpasses cellular scavenging capability (Reddy *et al.*, 2004), resulting in transitory excess of ROS, referred to as oxidative stress (Couée *et al.*, 2006). Under stress conditions, ROS generation leads to increased photorespiration, mitochondrial electron transport activity and fatty acid oxidation (Quan *et al.*, 2008; Sharma *et al.*, 2012). In fact, the closure of stomata because of water stress (diminishing CO<sub>2</sub> concentration in leaf mesophyll tissue) results in an accumulation of nicotinamide adenine dinucleotide phosphate (NADPH) and a decrease in NADP content. Oxygen operates as an alternate acceptor of electrons, forming the superoxide radical (O<sub>2</sub><sup>-</sup>) and H<sub>2</sub>O<sub>2</sub>, a reduction product of O<sub>2</sub> and the hydroxyl radical (OH), which is produced by the Haber–Weiss reaction (Cadenas, 1989). Active oxygen species can provoke lipid peroxidation and consequentially membrane damage, enzyme inactivation, protein degradation, pigment bleaching and disruption of DNA strands (Imlay and Linn, 1988; Sairam and Saxena, 2000). In field conditions, water deficit is often associated with high irradiance, and it was suggested that high irradiance stress can cause additional damage to the plant, limiting crop productivity (Behera *et al.*, 2002). The relationship between soil water availability, grapevine water stress and stem water potential (or stomatal conductance) was widely described in the literature (Williams and Araujo, 2002; Williams and Baeza, 2007; Suter *et al.*, 2019). In brief, plant water transport follows four steps: (i) soil to root; (ii) root to shoot xylem; (iii) shoot to leaf through the petiole; (iv) leaf to the atmosphere through stomata. General plant water status depends on water potential in soil layers close to the root system, evaporative demand and canopy dimension. Internal vine water deficits fix xylem sap flow to leaf transpiration in relation to soil water availability (Chone *et al.*, 2001).

Drought and high temperatures are perceived as crucial challenges for viticulture, threatening the ascertained connection between the local microclimate, local wine grape cultivars and the representative wine styles within terroirs (Irimia *et al.*, 2018). The impact of this change on the grapevine is important; in fact, research over the last decades has demonstrated modification of vine phenology (Sánchez-Gómez *et al.*, 2020), an enhancement in must alcoholic potential with a decrease in total acidity (Jones and Davis *et al.*, 2000), less predictable dimension and quality of grape yields (Van Leeuwen and Destrac-Irvine, 2017), unbalanced ripening of berries with associated colour and aroma profile alterations (Van Leeuwen and Darriet, 2016; Lu *et al.*, 2022; Torres *et al.*, 2022) and modification of characteristic wine sensory templates (Jones and Alves, 2012; Delrot *et al.*, 2020).

This change could lead to alterations in both the variety of grapes cultivated and the location of suitable viticultural areas, necessitating cooler and higher altitude sites (Moriondo *et al.*, 2013). Alternative solutions to this invasive displacement of wine-growing areas should take the form of sustainable vineyard management strategies also applied systemically throughout a company.

Natural zeolites are mentioned as ‘the magic rock’ by mineralogists (Eroglu *et al.*, 2017) due to their numerous uses such as

dietary integration in animal feeds (Behin *et al.*, 2019), soil improvers in agronomy (Hamid *et al.*, 2019), insecticide or pesticides used for plant safeguarding (Abdelgaleil *et al.*, 2022; Singh *et al.*, 2022), components in radioactive waste site remediation and decontamination (Gupta *et al.*, 2021) and additives to water and soil for wastewater treatment (Bayuo *et al.*, 2022). Zeolites support agricultural productivity and directly influence the quality of food products (Elemike *et al.*, 2019).

The properties that are structure-related include the high potency of hydration (Depmeier, 2009), extensive porosity (Boettinger and Ming, 2002), low density when dehydrated (Sacerdoti, 2007), crystal structure stability when dehydrated (Di Iorio *et al.*, 2019), cation exchange capacity (Baek *et al.*, 2018), homogenous molecular-sized channels (Li and Pidko, 2019), electrical conductivity (Waqas *et al.*, 2019), adsorption of gases and vapours (Bellat *et al.*, 2019) and catalytic properties (Feliczak-Guzik, 2018).

This experiment was designed to improve the vegetative and productive performance of the plant by adding zeolite to the composted soil. The product called Zeowine was created to interact with the nutritional and water efficiency of the plant. For these reasons, this project aimed to verify if the treatment with zeolite was able to positively influence the ecophysiology of the vine by promoting a greater tolerance to drought and significant light radiation. To achieve these objectives, a comparison was drawn between vines treated with zeolite and compost (Zeowine), vines treated with compost only and untreated vines (control) on Sangiovese cv. in an open field (*Vitis vinifera* L.). This experimentation is linked to the European project ZeoWine (co-financed by the European Commission under the LIFE Program 2014–2020 – Environment and Resources Efficiency). It was specifically chosen to evaluate the effects of the product in two different wine-growing areas in order to exclude the ‘terroir effect’ (Montalcino and San Miniato). The final results of the vineyard located in San Miniato were published separately to give prominence to both viticultural areas in equal measure (Cataldo *et al.*, 2023). The adopted procedures and tools being, the same throughout the Life Zeowine project, were the same for the two different trials. In addition, the effect of control (control, no treatment applied) treatment alone was also evaluated in the trial in Montalcino (not considered in the other vineyard). Finally, rootstock, clone, vineyard and the management of the vineyard itself are different: organic in Montalcino and biodynamic in the San Miniato Estate.

## Materials and methods

### Location, experimental project and composting process

The trial was organized at Col d’Orcia Estate (Lat 43°06’ N – Long 11°55’ E) (CdO), Italy. The estate is located on the southern slope of the Montalcino territory and is an integral part of the Orcia Valley. The Val d’Orcia is a unique territory that was declared part of the Patrimony of Humanity in the year 2004 (UNESCO).

The site benefits from an extremely favourable south-facing position with a protective barrier provided by Mount Amiata (1,750 m) against meteorological events such as floods or hail, and a mild climate influenced by the Tyrrhenian coast in the west, where the sea is some 35 km away. The climate is typically Mediterranean with limited rainfalls concentrated in the months of March, April, November and December.

The experiment was executed on 16-year-old organic vines (*V. vinifera* L., 1753) in two plant cultivation vintages (i.e. 2021

and 2022). The plants taken into consideration are red Sangiovese cultivar (clonal selection SG-CDO-6), on 161-49 C rootstock (*Vitis berlandieri* × *Vitis riparia*). The non-irrigated vineyard is located on a south-facing, moderate longitudinal slope at 6%, with North-South oriented rows and vines trained to upward vertical shoot positioning pruned to spur cordon at a spacing of 2.3 m (inter-row) × 1.0 m (intra-row) for a density of 4350 vines/ha. Each vine had a bud-load of about eight nodes. Standard Tuscan regional protocol for organic viticulture was implemented in all the trial years. The canopy was mechanically trimmed once shoots outgrew the top foliage wire.

From the analysis of the company's soil, a clayey-calcareous soil with the presence of a rocky skeleton emerges (clay 40.5%; sand 27.6%; silt 31.9%; active limestone 173 g/kg; pH 8.3; CSC 27.3 meq/100 g; organic matter 1.7%).

Using a randomized block design with ten replications per treatment (each replication was made by two contiguous inter-rows; on the middle row the measurements were taken from one selected grapevine), the comparison between the control, compost and Zeowine was set up. Ten experimental vines per treatment were then randomly identified and assumed as sub-replicates for the entire trial duration. On test vines, healthy and mature leaves inserted at median shoot level (3–4th node) were chosen for measurements.

Zeowine is a product made by combining the properties of zeolite (clinoptilolite) with the stable organic substance of a compost obtained on a company scale from the reuse of processing waste from grapes, pomace and stalks with the following characteristics: 8.26 pH, 45.9 C mol c/kg CSC, 25.68 C% TOC, 17.35 C/N, 73 mg/kg N-NO<sub>3</sub>, 611 mg/kg N-NH<sub>4</sub>, 317 mg K/kg available K and 328 mg P/kg available P (Doni *et al.*, 2021).

CdO provided material from grape skins, stalks and vineyard pruning waste, which were shredded to 4–5 cm and processed for their composting. The compost had the following characteristics: 7.37 pH, 36.4 C mol c/kg CSC, 27.01 C% TOC, 21.1 C/N, 196 mg/kg N-NO<sub>3</sub> and 469 mg/kg N-NH<sub>4</sub>. The optimal dimensions and typology of the zeolite (Zeocel Italia, PI, Italy) to be used for the production of Zeowine has been selected (85% clinoptilolite) with a granulometry of 0.2–2.5 mm identified in order to ensure better aeration of the heaps during composting. The application of treatments was executed on 1.6 ha of vineyard in production (February 2021 and 2022) with a manure spreader: Zeowine 30 t/ha and compost 20 t/ha (Doni *et al.*, 2021).

The agro-meteorological system Pre-meteo (Mybatec S.R.L., NO, Italy), situated near the vineyard (Montalcino, Italy), gathered the main parameters such as rainfall (mm) and air temperatures (°C).

### Leaf gas exchange, chlorophyll fluorescence, water potential (stem) and leaf temperature

Ecophysiological surveys (between 11:10 a.m. and 13:10 p.m.) were conducted on ten replicates per treatment (on tagged vines) every week, from May to harvest: in 2021, 31 May, 15–30 June, 15–30 July, 17–30 August and 9–13 September; in 2022, 31 May, 17 June, 4–25 July, 3–17–26 August and 10–19 September. Data were collected for following parameters: °C (leaf temperature), PN (photosynthesis), gs (stomatal conductance) and E (transpiration), using the Ciras 3PP Systems gas analyser, USA (–400 ppm CO<sub>2</sub>, surrounding temperature IR Thermometry, RGBW control 38%, 37%, 25%, 0% and 1300 μmol/m<sup>2</sup>/s photon flux) (Salvi *et al.*, 2020). Extrinsic water use efficiency (eWUE) was

estimated from the photosynthesis/transpiration ratio (Poni *et al.*, 2014). On the same leaves between 13:15 and 14:15 p.m., stem mid-day water potential (Ψ<sub>stem</sub>) was evaluated using a Scholander pressure chamber (600-type, PMS Instrument Co, Albany, OR, USA) (Chone *et al.*, 2001). The method consists of increasing the pressure around a leaf petiole until xylem sap appears at the cut end of the petiole, which extends outside the Scholander chamber and is exposed to atmospheric pressure (Boyer, 1967). The surveys were conducted on the tagged vines every week at the beginning of the summer period, from June to harvest: in 2021, 30 June, 17–30 July, 17–30 August and 9–13 September; in 2022, 4–25 July, 3–17–26 August and 10–19 September.

On the same days, chlorophyll fluorescence was assessed with a Handy-PEA fluorometer (Handy-PEA®, UK) on leaves adapted to the dark for ~30 min (Christen *et al.*, 2007).

### Technological parameters of berries

In each treatment (Zeowine, compost and control), 100 berries per replication were casually sampled to analyse technological maturity. The sample of 100 berries (ten berries for each tagged vine) was collected from the tagged vines. The berries were sampled from different areas of the bunch: central, upper, lower and lateral parts. Firstly, the berries of each treatment were individually weighed with the Kern PCD model (a precision-digital scale). The sample was crushed to analyse sugar content (° Brix), total acidity (g/l tartaric acid) and pH of the must. The following tools and products were employed for technological analysis: a portable-optical refractometer (RHA-503), a pH meter (HHTEC), bromothymol blue, glass burettes and sodium hydroxide solution (NaOH-0.1 M). In each treatment (Zeowine, compost and control), a further 100 berries per replication were casually sampled to analyse phenolic maturity. Total and extractable anthocyanins were estimated by Glories' method (Kontoudakis *et al.*, 2010). The determination of nine major anthocyanins (cyanidin-3-glucoside, delphinidin-3-glucoside, malvidin-3-acetylglucoside, malvidin-3-cumarylglucoside, malvidin-3-glucoside, peonidin-3-acetylglucoside, peonidin-3-cumarylglucoside, peonidin-3-glucoside and petunidin-3-glucoside) in musts was performed according to OIV-MA-AS315-11: R2007 1 Method OIV-MA-AS315-11 Type II method HPLC-Determination, by an external laboratory (ISVEA), under the analysis conditions proposed by Resolution Oeno 22/2003 and revised in Oeno 12/2007 (OIV, 2021). In addition, with HPLC-HRMS (high-performance liquid chromatography-high resolution mass spectrometry) (Sun *et al.*, 2018) coumaric acid, gallic acid, caffeic acid, ferulic acid, kaempferol-3-O-glucoside, quercetin-3-O-glucoside, quercetin-3-O-rutinoside, quercetin-3-O-galactoside and quercetin-3-O-glucuronide were evaluated. Berry samples were preserved at –80°C until the moment of analysis. The determination of yeast-assimilable nitrogen (as the sum of amino and ammoniacal nitrogen) in musts was performed with an enzymatic-colorimetric kit (Steroglass, Pg, Italy) (Suriñach Ros, 2017).

Finally, on tagged vines, the number of clusters per vine, the weight of bunch per vine and total yield/vine were determined at harvest with a digital scale (VAR model, Italy).

### Statistical analysis

Data and graphs were processed with R and RStudio (R Development Core Team) (4.0.3. version) (Tidyverse packages; Lee *et al.*, 2020) with one-way analysis of variance (ANOVA)

( $P \leq 0.05$ ). Means comparison was performed by the Tukey HSD test (Abdi and Williams, 2010) ( $P \leq 0.05$ ).

## Results

### Meteorological parameters

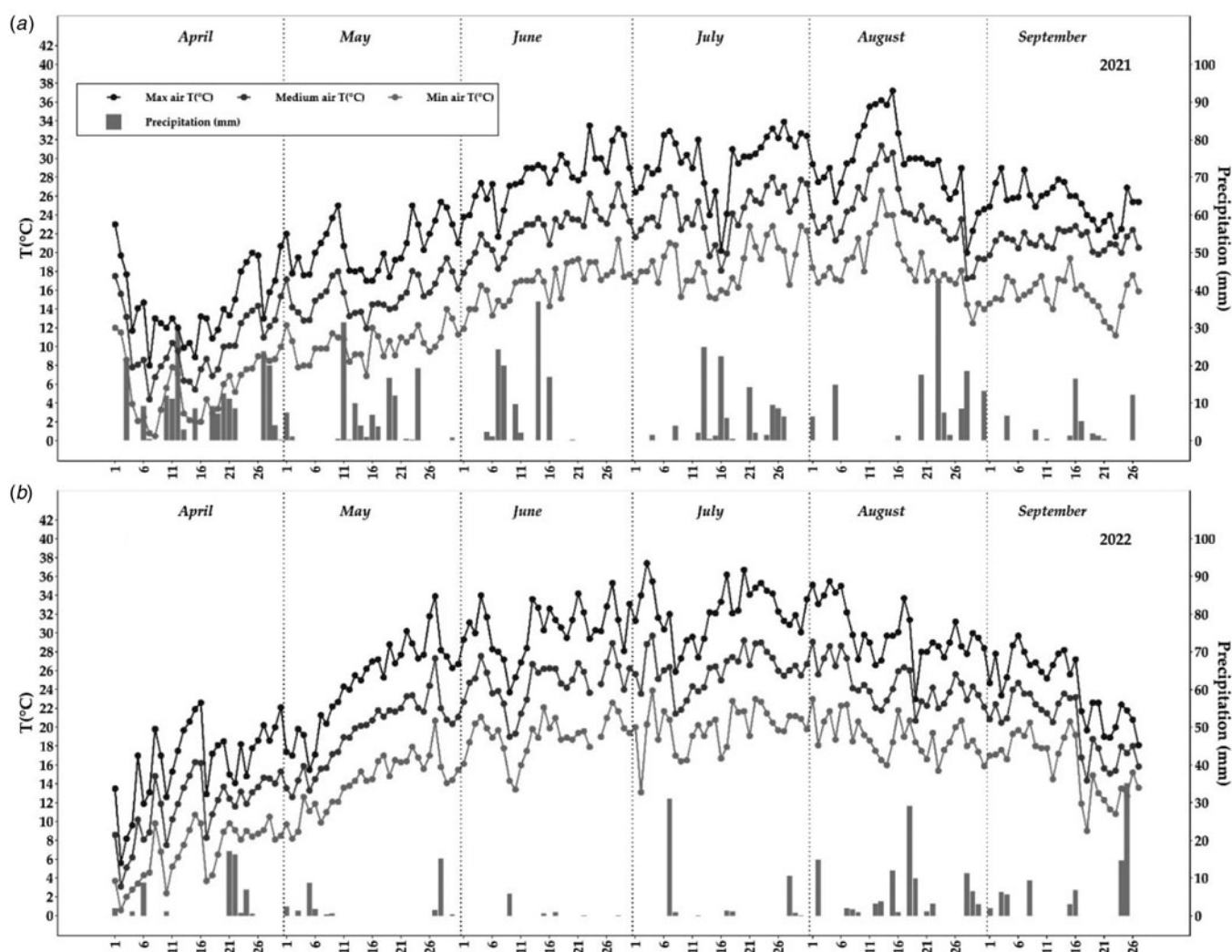
The 2021–2022 climate situation of the experimental area is reported in Fig. 1. Daily minimum, average and maximum air temperatures were recorded in both seasons 2021–2022 (from April to September). The 2022 season was more arid and less rainy from April to August than in 2021. The rains in 2022 were mainly concentrated in the month of September. The rainfall summation was: 193.20 mm in April 2021, 116.1 mm in May 2021, 114.0 mm in June 2021, 105.6 mm in July 2021, 131.1 mm in August 2021 and 51.8 mm in September 2021; 54.6 mm in April 2022, 32.3 mm in May 2022, 7.80 mm in June 2022, 46.3 mm in July 2022, 103.7 mm in August 2022 and 112.4 mm in September 2022. In 2022, rainfall was concentrated in the final phase, late August and September. The monthly averages of max temperatures from April to September were: 14.5, 20.6,

28.3, 29.8, 29.4 and 25.4°C (2021); 16.4, 27.6, 30.4, 32.8, 30.1 and 23.8°C (2022). The days where temperatures exceeded 35°C were the following: in 2021, on August 11 at 35.5°C, on August 12 at 35.8°C, on August 13 at 36.2°C, on August 14 at 35.7°C, on August 15 at 37.2°C; in 2022, on July 3 at 37.4°C, on July 4 at 35.5°C, on July 17 at 36.2°C, on July 20 at 36.7°C, on July 23 at 35.3°C, on August 1 at 35.1°C and on August 4 at 35.5°C.

### Leaf gas exchange, chlorophyll fluorescence, stem water potential and leaf temperature

The grapevine ecophysiological parameters according to three different land conductions (Zeowine, compost and control) are indicated in Tables 1 and 2, and Figs 2 and 3.

Significant differences in leaf temperatures, eWUE and transpiration were found, particularly during the hottest periods. Essentially no differences were seen between compost and control treatment. During the hottest periods in the 2021 season, the leaf temperature underwent a decrease of 2.66% in Zeowine compared to compost and a decrease of 4.19% compared to control (June 15); a 3.64% decrease in Zeowine *v.* compost and a 4.59% decrease



**Figure 1.** Meteorological parameters of the experiment location (Col d'Orcia, Montalcino, Italy). Monthly averages of mean, maximum and minimum air temperature (°C) and monthly total precipitation (mm) were measured from April to September (2021–2022 seasons).

**Table 1.** Ecophysiological parameters – 2021 season

| 2021         | Transpiration (mmol/m <sup>2</sup> s) |               |               | Water use efficiency (μmol/mmol) |                |                | Leaf temperature (°C) |                |                 | Fluorescence of chlorophyll (Fv/Fm) |               |               |
|--------------|---------------------------------------|---------------|---------------|----------------------------------|----------------|----------------|-----------------------|----------------|-----------------|-------------------------------------|---------------|---------------|
|              | Zeowine                               | Compost       | Control       | Zeowine                          | Compost        | Control        | Zeowine               | Compost        | Control         | Zeowine                             | Compost       | Control       |
| 31 May       | 1.33 ± 0.28 a                         | 1.26 ± 0.17 a | 1.24 ± 0.43 a | 7.85 ± 0.98 a                    | 6.43 ± 1.24 b  | 7.42 ± 2.46 ab | 23.51 ± 0.97 a        | 24.23 ± 0.73 a | 23.92 ± 0.54 a  | 0.82 ± 0.04 a                       | 0.82 ± 0.01 a | 0.82 ± 0.03 a |
| 15 June      | 4.81 ± 1.54 a                         | 4.83 ± 0.77 a | 4.93 ± 1.26 a | 3.20 ± 1.14 a                    | 2.12 ± 0.97 b  | 1.96 ± 0.73 b  | 33.14 ± 1.37 b        | 34.02 ± 0.64 a | 34.53 ± 0.87 a  | 0.82 ± 0.02 a                       | 0.80 ± 0.02 a | 0.80 ± 0.02 a |
| 30 June      | 5.98 ± 0.98 c                         | 7.34 ± 0.74 b | 7.91 ± 0.83 a | 1.85 ± 0.44 a                    | 0.91 ± 0.30 b  | 0.79 ± 0.34 b  | 31.83 ± 1.31 b        | 32.99 ± 0.52 a | 33.29 ± 0.53 a  | 0.76 ± 0.03 a                       | 0.74 ± 0.04 a | 0.74 ± 0.03 a |
| 15 July      | 4.26 ± 0.43 b                         | 4.91 ± 0.39 a | 5.06 ± 1.08 a | 2.80 ± 0.58 a                    | 2.13 ± 0.48 b  | 1.93 ± 0.52 b  | 26.50 ± 1.29 b        | 28.88 ± 0.90 a | 28.79 ± 1.49 a  | 0.71 ± 0.07 a                       | 0.70 ± 0.07 a | 0.68 ± 0.15 a |
| 30 July      | 3.19 ± 0.68 c                         | 4.98 ± 0.76 a | 4.13 ± 0.71 b | 3.23 ± 0.60 a                    | 1.64 ± 0.30 b  | 2.00 ± 0.53 b  | 33.49 ± 0.51 b        | 34.05 ± 0.23 a | 34.16 ± 0.56 a  | 0.80 ± 0.02 a                       | 0.77 ± 0.02 b | 0.75 ± 0.03 b |
| 17 August    | 2.40 ± 0.56 b                         | 2.68 ± 0.53 b | 3.43 ± 0.55 a | 4.15 ± 0.67 a                    | 2.71 ± 0.39 b  | 1.97 ± 0.31 c  | 32.27 ± 0.86 c        | 33.08 ± 0.69 b | 33.88 ± 0.68 a  | 0.75 ± 0.03 a                       | 0.67 ± 0.13 b | 0.68 ± 0.09 b |
| 30 August    | 1.90 ± 0.13 a                         | 1.90 ± 0.26 a | 1.91 ± 0.31 a | 4.39 ± 0.79 a                    | 3.72 ± 1.08 ab | 3.57 ± 0.76 b  | 24.22 ± 0.88 c        | 25.19 ± 0.92 b | 25.82 ± 0.64 a  | 0.79 ± 0.02 a                       | 0.70 ± 0.05 b | 0.70 ± 0.06 b |
| 9 September  | 2.38 ± 0.34 a                         | 2.16 ± 0.29 a | 2.08 ± 0.27 a | 2.91 ± 1.01 a                    | 2.11 ± 1.00 ab | 2.10 ± 1.36 b  | 25.34 ± 1.40 b        | 26.13 ± 2.56 a | 26.59 ± 1.34 a  | 0.79 ± 0.02 a                       | 0.77 ± 0.03 a | 0.78 ± 0.03 a |
| 13 September | 2.06 ± 0.48 a                         | 1.77 ± 0.52 a | 1.65 ± 0.56 a | 3.45 ± 0.96 a                    | 2.84 ± 1.05 b  | 2.87 ± 0.77 ab | 24.65 ± 0.34 b        | 25.63 ± 1.33 a | 25.22 ± 1.12 ab | 0.73 ± 0.02 a                       | 0.69 ± 0.05 a | 0.71 ± 0.06 a |

Transpiration (E), extrinsic water use efficiency (eWUE), leaf temperature (°C) and fluorescence of chlorophyll of *Vitis vinifera* with three different soil managements treated. Measurements were conducted from May 2021 to September 2021. Data (mean ± s.e., n = 10) were subjected to one-way ANOVA. Different letters indicate significant differences among Zeowine, compost and control (LSD test, P ≤ 0.05).

**Table 2.** Ecophysiological parameters – 2022 season

| 2022         | Transpiration (mmol/m <sup>2</sup> s) |               |               | Water use efficiency (μmol/mmol) |                |               | Leaf temperature (°C) |               |               | Fluorescence of chlorophyll (Fv/Fm) |               |               |
|--------------|---------------------------------------|---------------|---------------|----------------------------------|----------------|---------------|-----------------------|---------------|---------------|-------------------------------------|---------------|---------------|
|              | Zeowine                               | Compost       | Control       | Zeowine                          | Compost        | Control       | Zeowine               | Compost       | Control       | Zeowine                             | Compost       | Control       |
| 31 May       | 1.88 ± 0.23 a                         | 2.01 ± 0.29 a | 2.00 ± 0.32 a | 5.71 ± 1.22 a                    | 4.95 ± 1.26 a  | 5.13 ± 1.77 a | 23.3 ± 0.98 a         | 23.6 ± 0.63 a | 23.9 ± 0.23 a | 0.83 ± 0.02 a                       | 0.82 ± 0.03 a | 0.82 ± 0.03 a |
| 17 June      | 3.61 ± 1.21 a                         | 4.03 ± 1.05 a | 4.16 ± 1.10 a | 3.18 ± 0.32 a                    | 2.85 ± 0.68 a  | 2.36 ± 0.83 a | 29.2 ± 0.89 b         | 33.6 ± 0.68 a | 34.0 ± 0.67 a | 0.80 ± 0.03 a                       | 0.79 ± 0.02 a | 0.78 ± 0.02 a |
| 4 July       | 6.28 ± 1.13 b                         | 8.33 ± 0.90 a | 7.99 ± 1.22 a | 1.45 ± 0.89 a                    | 0.78 ± 0.63 b  | 0.65 ± 0.74 b | 34.0 ± 1.21 b         | 38.3 ± 0.43 a | 38.6 ± 0.89 a | 0.77 ± 0.03 a                       | 0.74 ± 0.03 b | 0.73 ± 0.03 b |
| 25 July      | 7.08 ± 0.88 b                         | 9.23 ± 0.45 a | 9.12 ± 0.54 a | 1.54 ± 0.32 a                    | 0.78 ± 0.87 b  | 0.78 ± 0.56 b | 34.0 ± 1.14 b         | 37.4 ± 1.05 a | 37.0 ± 1.25 a | 0.71 ± 0.07 a                       | 0.68 ± 0.02 b | 0.66 ± 0.04 b |
| 3 August     | 4.14 ± 0.66 b                         | 6.42 ± 0.58 a | 6.96 ± 0.91 a | 2.30 ± 1.21 a                    | 1.26 ± 0.99 b  | 1.07 ± 0.87 b | 33.3 ± 0.62 b         | 35.1 ± 0.77 a | 35.2 ± 0.50 a | 0.82 ± 0.01 a                       | 0.78 ± 0.02 b | 0.76 ± 0.01 b |
| 17 August    | 4.04 ± 0.36 a                         | 4.36 ± 0.67 a | 4.77 ± 0.54 a | 2.33 ± 1.03 a                    | 1.44 ± 1.08 b  | 1.15 ± 0.75 b | 32.1 ± 0.23 a         | 33.1 ± 0.44 a | 33.0 ± 0.38 a | 0.81 ± 0.02 a                       | 0.77 ± 0.04 b | 0.78 ± 0.02 b |
| 26 August    | 3.25 ± 0.17 a                         | 3.12 ± 0.24 a | 3.53 ± 0.22 a | 3.07 ± 1.06 a                    | 2.20 ± 1.05 ab | 1.37 ± 0.71 b | 25.1 ± 0.95 b         | 27.2 ± 0.75 a | 26.9 ± 0.32 a | 0.79 ± 0.02 a                       | 0.77 ± 0.05 a | 0.78 ± 0.06 a |
| 10 September | 2.27 ± 0.24 a                         | 2.13 ± 0.27 a | 2.32 ± 0.23 a | 3.67 ± 1.08 a                    | 3.12 ± 1.02 a  | 1.88 ± 1.10 b | 23.2 ± 1.11 b         | 25.5 ± 0.54 a | 25.3 ± 1.04 a | 0.80 ± 0.02 a                       | 0.79 ± 0.01 a | 0.79 ± 0.03 a |
| 19 September | 1.99 ± 0.28 a                         | 1.87 ± 0.33 a | 2.03 ± 0.44 a | 3.59 ± 1.13 a                    | 3.74 ± 1.08 a  | 3.39 ± 1.00 a | 22.3 ± 0.64 a         | 22.8 ± 0.54 a | 22.2 ± 0.67 a | 0.79 ± 0.02 a                       | 0.77 ± 0.03 a | 0.77 ± 0.04 a |

Transpiration (E), extrinsic water use efficiency (eWUE), leaf temperature (°C) and fluorescence of chlorophyll of *Vitis vinifera* with three different soil managements treated. Measurements were conducted from May 2022 to September 2022. Data (mean ± s.e., n = 10) were subjected to one-way ANOVA. Different letters indicate significant differences among Zeowine, compost and control (LSD test, P ≤ 0.05).

v. control was also demonstrated on June 30 (Table 1). During the hottest periods of the 2022 season, the leaf temperature underwent a decrease of 12.6% in Zeowine compared to compost and a decrease of 13.4% compared to control (July 4); a 10.1% decrease in Zeowine v. compost and an 8.79% decrease v. control was also demonstrated on July 25 (Table 2).

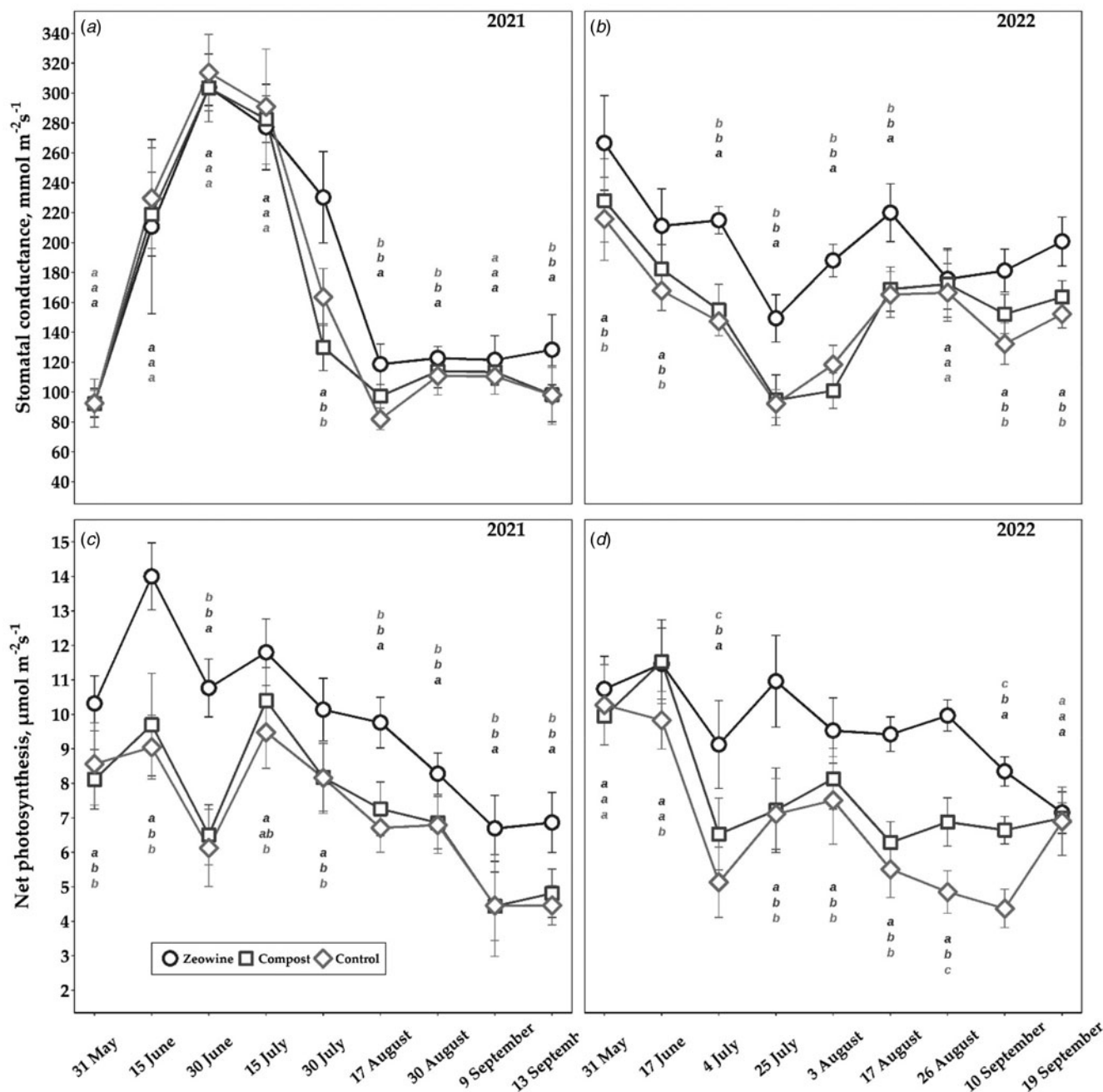
Significant differences in net photosynthesis and stomatal conductance during seasons were found (Fig. 2). Generally, no differences were ever found between the compost and the control treatments. The Zeowine treatment showed better values than the other two treatments, especially during the hottest periods. In general, the trends of photosynthesis and conductance

reflect the climatic situation showing reductions in the driest periods.

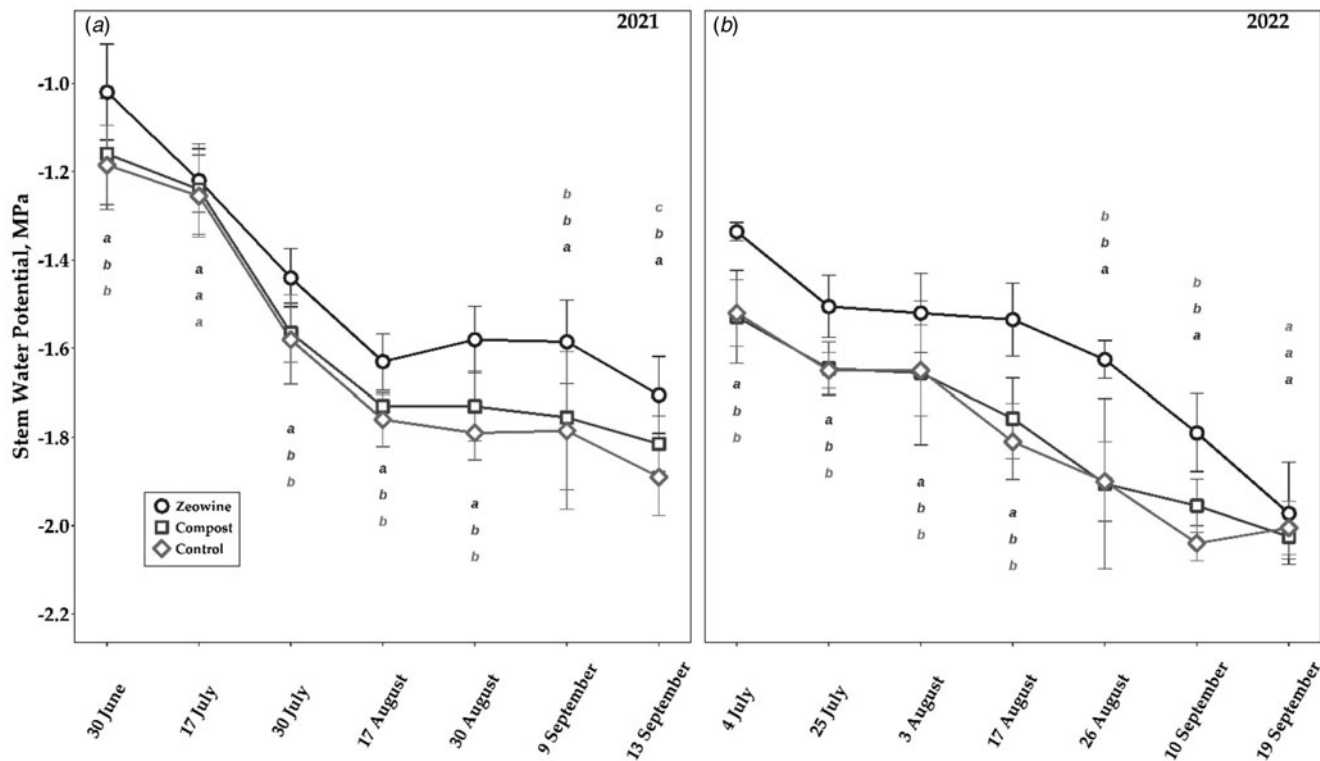
During the moments of decline in water potential, the compost and the control recorded a decrease of 9.49 and 13.3%, respectively, compared to Zeowine (17 August 2021) and on 25 July 2022, there were decreases of 10.4 and 10.7%, respectively (Fig. 3).

### Grape composition and production

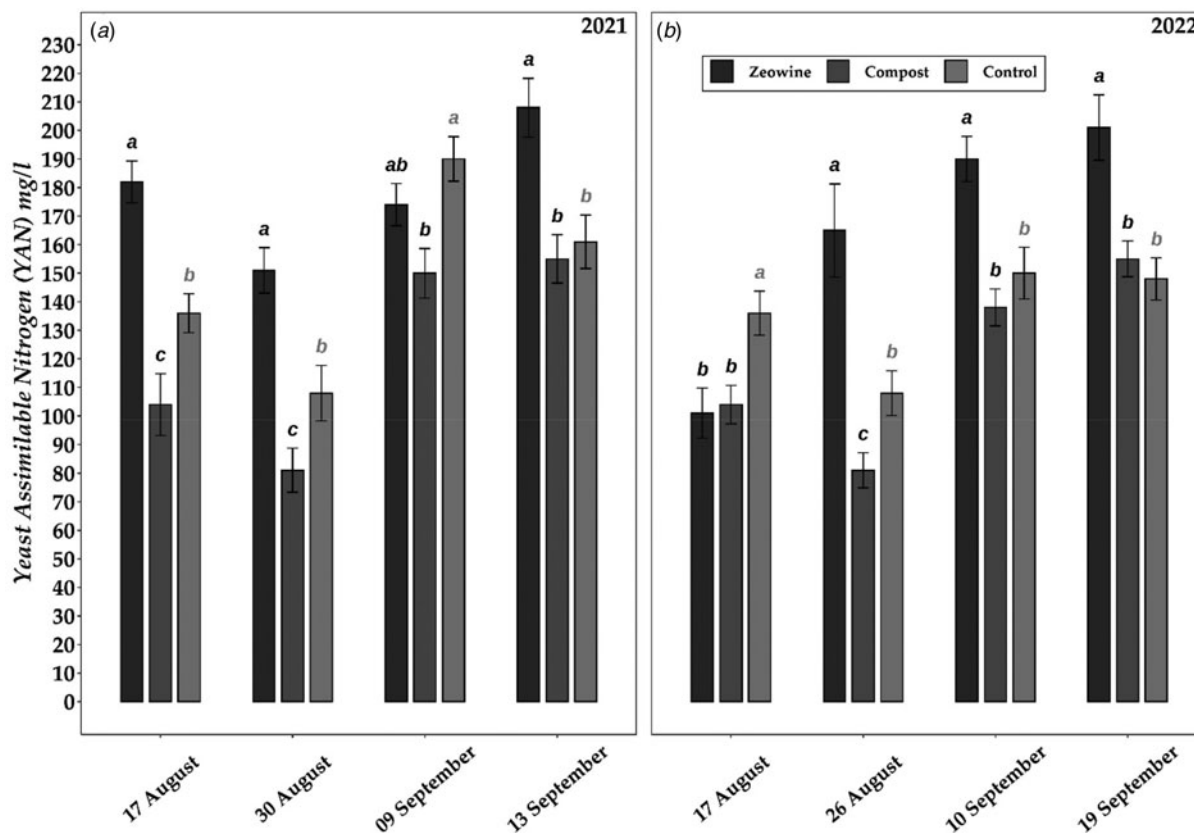
The treatment had a positive effect on the readily assimilable nitrogen content (Fig. 4). During the harvests (13th and 19th September), Zeowine recorded significantly higher values than



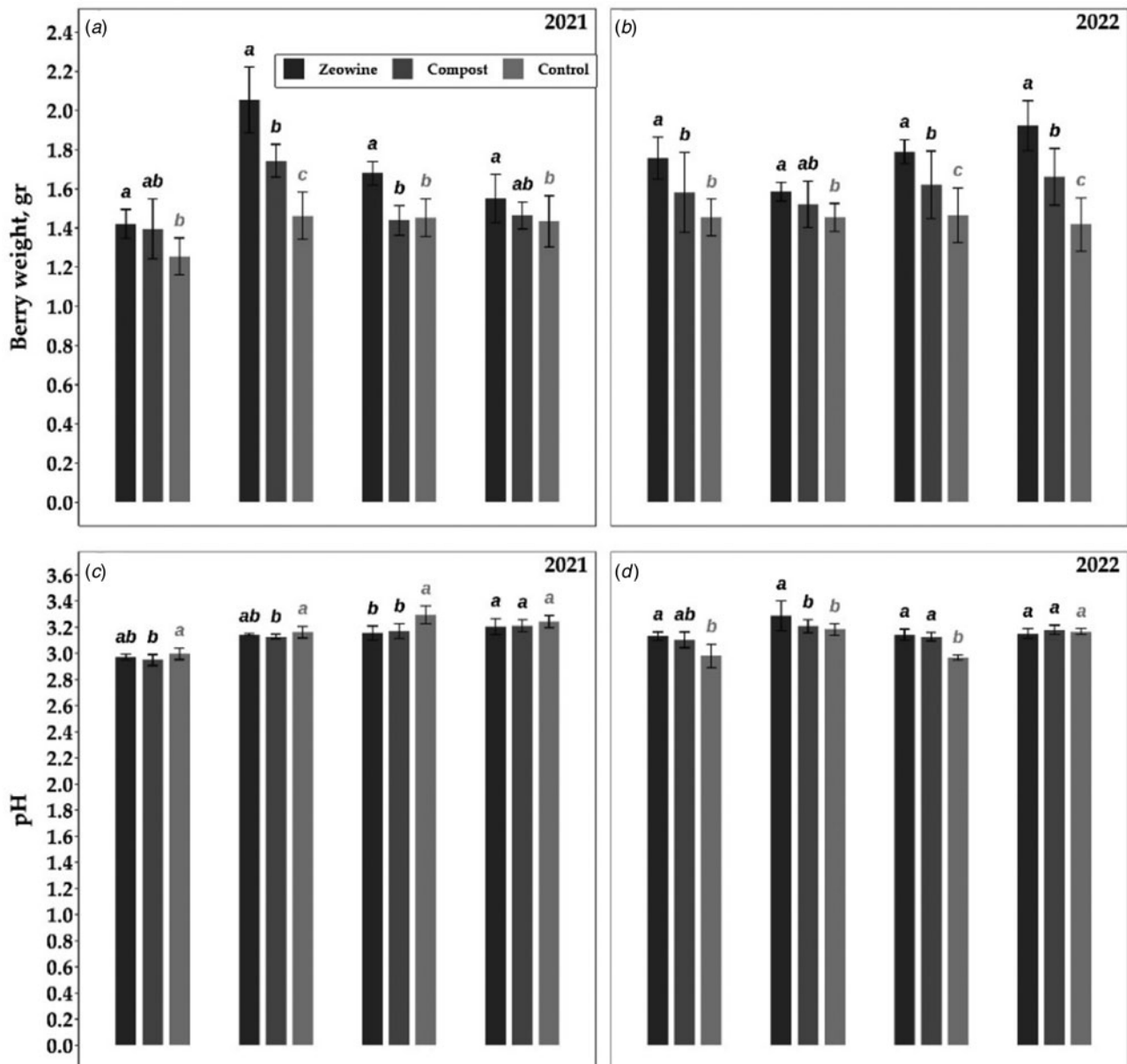
**Figure 2.** Ecophysiological parameters – 2021/2022 seasons. Net photosynthesis (PN) and stomatal conductance (gs) of *Vitis vinifera* with three-way different soil managements treated. Measurements were conducted from May to September (2021 and 2022). Data (mean ± s.e., n = 10) were subjected to one-way ANOVA. The bars represent the standard deviation. Different letters indicate significant differences among Zeowine, compost and control (LSD test, P ≤ 0.05).



**Figure 3.** Ecophysiological parameters – 2021/2022 seasons. Stem water potential ( $\Psi_{\text{stem}}$ ) of *Vitis vinifera* with three different soil managements treated. Measurements were conducted from June to September (2021 and 2022). Data (mean  $\pm$  s.e.,  $n = 10$ ) were subjected to one-way ANOVA. The bars represent the standard deviation. Different letters indicate significant differences among Zeowine, compost and control (LSD test,  $P \leq 0.05$ ).



**Figure 4.** Yeast assimilable nitrogen (YAN). YAN of *Vitis vinifera* treated with Zeowine, compost and control during two seasons (2021–2022). Measurements were conducted four times: full veraison (17 August 2021 and 17 August 2022), mid maturation (30 August 2021 and 26 August 2022), full maturation (9 September 2021 and 10 September 2022), and harvest (13 September 2021 and 19 September 2022). Data (mean  $\pm$  s.e.,  $n = 10$ ) were subjected to one-way ANOVA. The bars represent the standard deviation. Different letters indicate significant differences among Zeowine, compost and control (LSD test,  $P \leq 0.05$ ).



**Figure 5.** Technological maturity. Sugar content (°Brix), total acidity (TA), pH and berry weight of *Vitis vinifera* treated with Zeowine, compost and control during two seasons (2021–2022). Measurements were conducted four times: full veraison (17 August 2021 and 17 August 2022), mid maturation (30 August 2021 and 26 August 2022), full maturation (9 September 2021 and 10 September 2022) and harvest (13 September 2021 and 19 September 2022). Data (mean  $\pm$  s.e.,  $n = 10$ ) were subjected to one-way ANOVA. The bars represent the standard deviation. Different letters indicate significant differences among Zeowine, compost and control (LSD test,  $P \leq 0.05$ ).

the other treatments. No difference in nitrogen content was noted in relation to vintage.

The zeolitic amendment proved to be effective in improving the technological ripening of the grapes. Significantly higher values were found in the weight of the berry and in the sugar content compared to the other two treatments. No difference was found in both years at the time of harvest in the pH of the must (Fig. 5).

During the anthocyanin accumulation period (from veraison to harvest), the zeolitic amendment showed higher values of total and extractable anthocyanins. In particular, in the harvests (13 and 19 September), the following increases of extractable anthocyanins in Zeowine against compost and control were

recorded: +8.26, +8.52%, and +32.4, +25.7%. Considering the ripening season during 2021, higher values of anthocyanins were recorded in all treatments compared to 2022 (Fig. 6).

No significant differences were found in the percentage of anthocyanins among treatments. caffeic, ferulic and coumaric acids were found in trace amounts or not detected. Zeowine grapes harvested in 2021 revealed significantly lower values of quercetin-3-O-glucoside, quercetin-3-O-galactoside and quercetin-3-O-glucuronide compared to compost and control. Furthermore, the same plants in 2022 detected significantly lower values of quercetin-3-O-glucoside, quercetin-3-O-glucuronide and quercetin-3-O-rutinoside compared to compost and control (Tables 2–4).



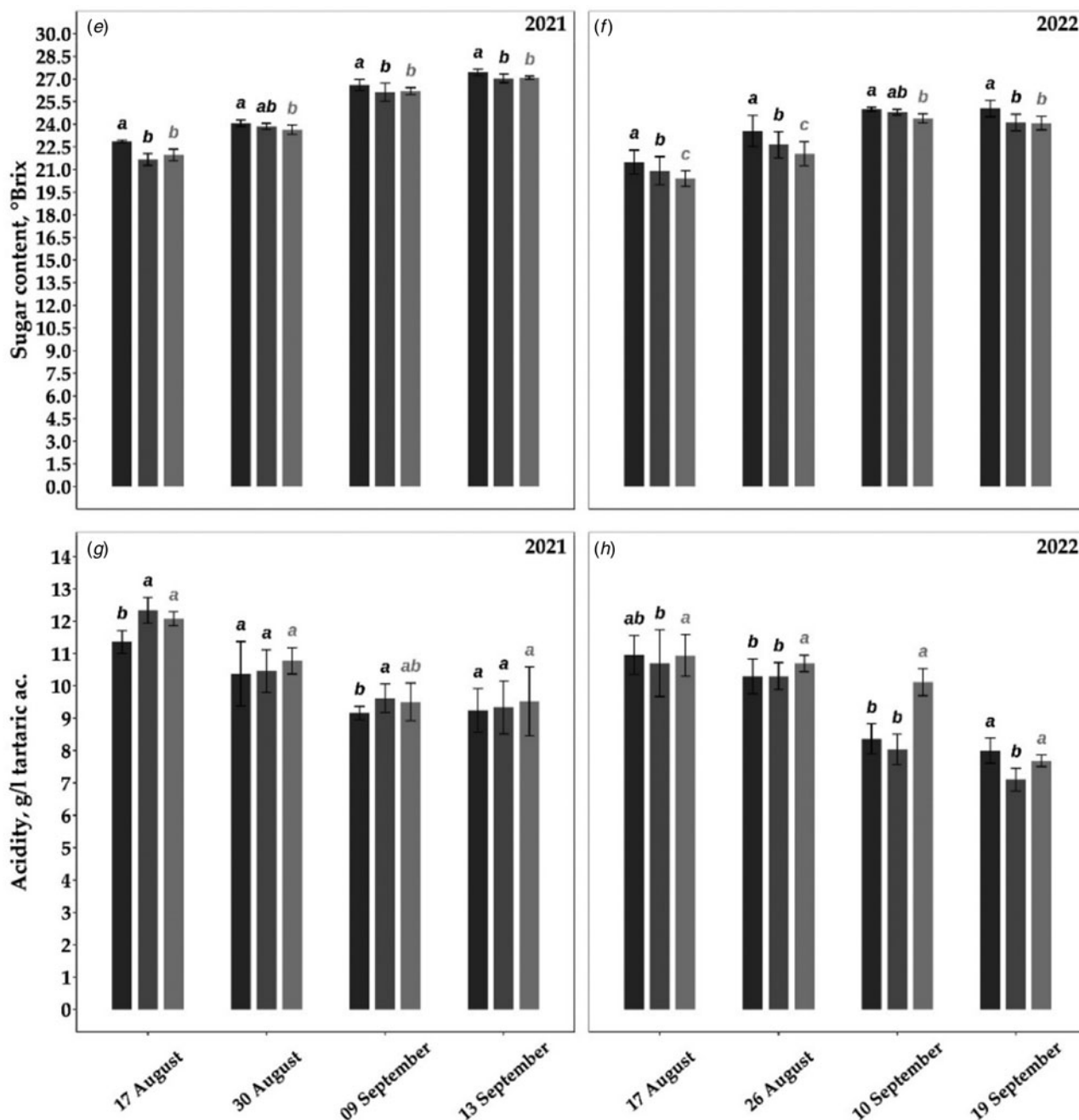


Figure 5. Continued.

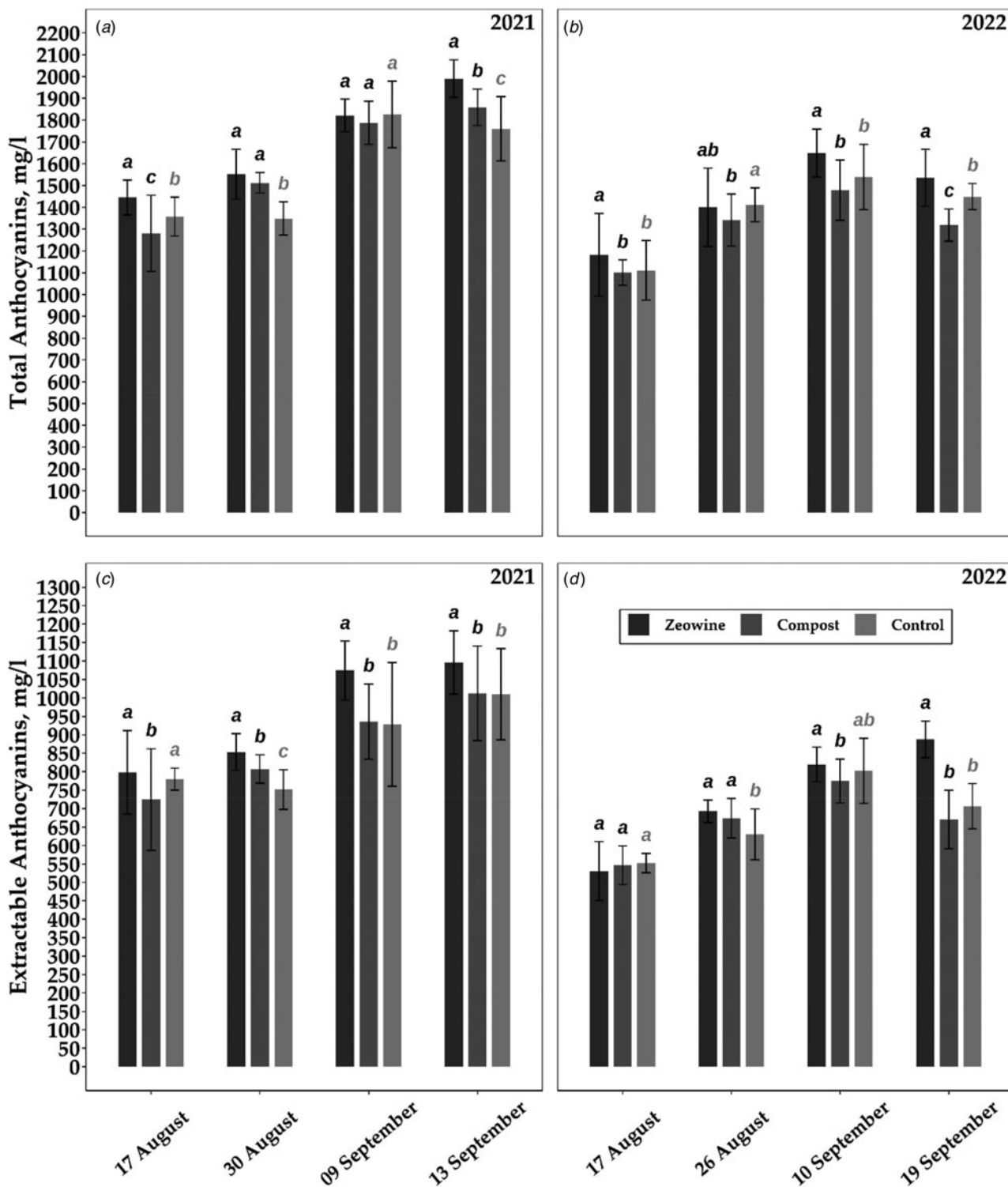
No differences were recorded in the number of bunches; however, both the production and the weight of the bunch showed higher values in the Zeowine treatment (Fig. 7).

### Discussion

In our study  $\Psi$  stem, PN and gs were significantly strengthened and improved by zeolite addition in ZEOWINE grapevines with respect to compost and control. The Zeowine treatment positively influenced water stress. In fact, by valorising the soil chemical-physical features, such as potential infiltration, hydraulic conductivity and water-holding skill (Xiubin and Zhanbin, 2001), several authors reported that water stress in plants can be alleviated using

zeolite reinforced with substances of natural origin such as in *Aloe vera* L. (Hazrati et al., 2017), *Oryza sativa* L. (Zheng et al., 2018), *Trigonella foenum-graecum* L. (Baghbani-Arani et al., 2017) and *Malva sylvestris* L. (Ahmadi et al., 2015). These data confirmed what was observed in the parallel experimentation (San Miniato vineyard) (Cataldo et al., 2023); the reproducibility of the treatment effect (Zeowine) in a different terroir is considered fundamental data in the monitoring of a product (experimental treatments may interact with different environmental conditions) (Richter et al., 2009).

It is likely that in the compost and control grapevines, PN, and consequently eWUE, were restricted almost exclusively by impaired photochemistry (i.e. a decline in Fv/Fm ratio) due to



**Figure 6.** Total and extractable anthocyanins of *Vitis vinifera* treated with Zeowine, compost and control during two seasons (2021–2022). Measurements were conducted four times: full veraison (17 August 2021 and 17 August 2022), mid maturation (30 August 2021 and 26 August 2022), full maturation (9 September 2021 and 10 September 2022) and harvest (13 September 2021 and 19 September 2022). Data (mean ± s.e., n = 10) were subjected to one-way ANOVA. The bars represent the standard deviation. Different letters indicate significant differences among Zeowine, compost and control (LSD test, P ≤ 0.05).

the hottest period during the growing season (Flexas *et al.*, 2004). The need for ‘heat escape’ through transpiration cooling dictated by high-temperature stress (Araújo *et al.*, 2019; Sadok *et al.*, 2021) and the need for water conservation under water-limited

conditions (Ye *et al.*, 2020) was counterbalanced in the compost and control treatments by a tendential increase in transpiration and a reduction in stomatal conductance. On the contrary, the plants treated with Zeowine faced heat stress and water stress in

**Table 3.** Phenolic maturity

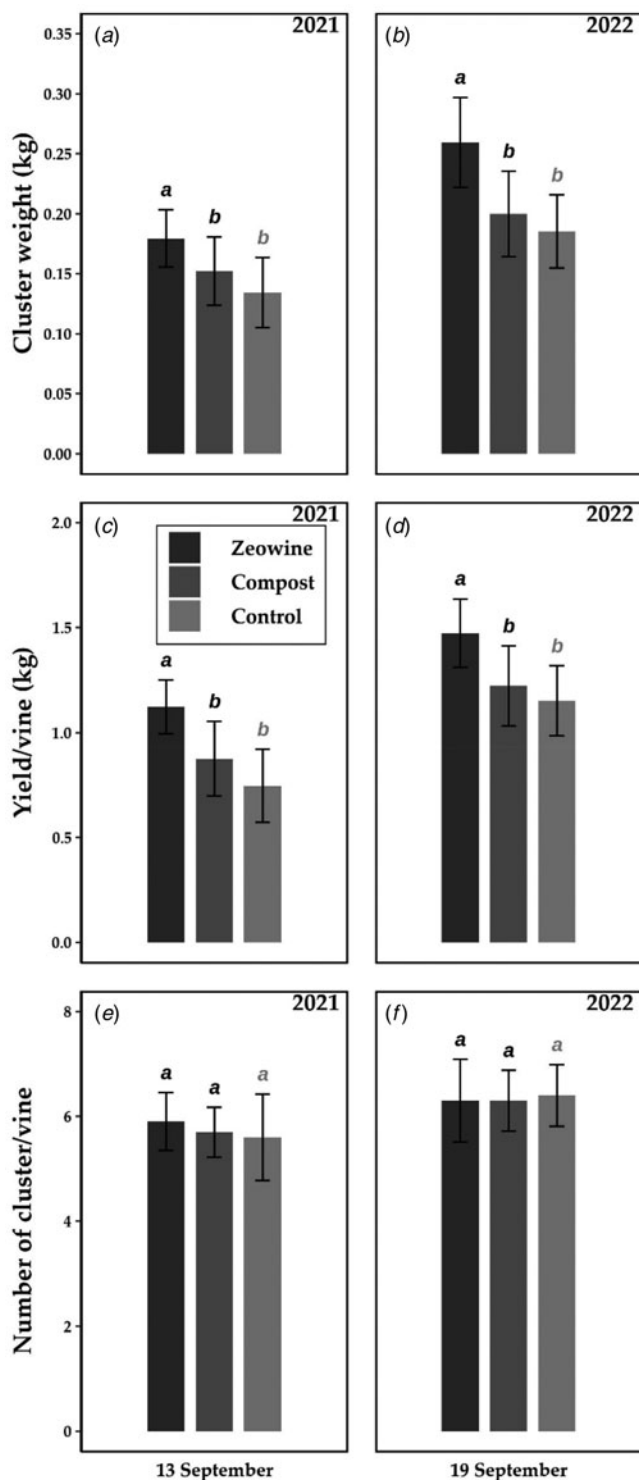
|                             | 17 August 2021 |                 |                | 30 August 2021 |                |                | 9 September 2021 |                |                | 13 September 2021 |                |                 | m.u.  |
|-----------------------------|----------------|-----------------|----------------|----------------|----------------|----------------|------------------|----------------|----------------|-------------------|----------------|-----------------|-------|
|                             | Zeowine        | Compost         | Control        | Zeowine        | Compost        | Control        | Zeowine          | Compost        | Control        | Zeowine           | Compost        | Control         |       |
| Cyanidin-3-glucoside        | 28.3 ± 3.15 a  | 27.7 ± 2.22 a   | 24.6 ± 3.11 a  | 18.9 ± 1.32 b  | 23.8 ± 2.47 a  | 24.4 ± 3.28 a  | 22.6 ± 3.25 a    | 21.8 ± 2.41 a  | 20.9 ± 2.82 a  | 17.9 ± 2.10 b     | 21.5 ± 2.33 b  | 29.6 ± 1.18 a   | %     |
| Delphinidin-3-glucoside     | 12.6 ± 1.24 a  | 13.6 ± 1.18 a   | 15.0 ± 2.05 a  | 17.4 ± 1.10 a  | 14.2 ± 2.31 a  | 16.6 ± 1.78 a  | 13.6 ± 2.62 a    | 13.2 ± 3.09 a  | 13.1 ± 2.48 a  | 14.7 ± 2.56 a     | 14.4 ± 2.54 a  | 13.0 ± 2.37 a   | %     |
| Malvidin-3-acetylglucoside  | <0.10 ± 0.00 a | <0.10 ± 0.00 a  | <0.10 ± 0.00 a | <0.10 ± 0.00 a | <0.10 ± 0.00 a | <0.10 ± 0.00 a | <0.10 ± 0.00 a   | <0.10 ± 0.00 a | <0.10 ± 0.00 a | <0.10 ± 0.00 a    | <0.10 ± 0.00 a | <0.10 ± 0.00 a  | %     |
| Malvidin-3-cumarylglucoside | <0.10 ± 0.00 a | <0.10 ± 0.00 a  | <0.10 ± 0.00 a | <0.10 ± 0.00 a | <0.10 ± 0.00 a | <0.10 ± 0.00 a | <0.10 ± 0.00 a   | 10.60 ± 2.74 a | <0.10 ± 0.00 a | <0.10 ± 0.00 a    | <0.10 ± 0.00 a | <0.10 ± 0.00 a  | %     |
| Malvidin-3-glucoside        | 26.5 ± 3.18 a  | 26.7 ± 4.14 a   | 28.8 ± 3.50 a  | 33.0 ± 3.27 a  | 29.6 ± 2.27 a  | 27.2 ± 2.41 a  | 30.1 ± 1.05 a    | 31.6 ± 3.88 a  | 32.4 ± 3.26 a  | 34.1 ± 3.27 a     | 30.8 ± 3.27 a  | 24.1 ± 2.89 b   | %     |
| Peonidin-3-acetylglucoside  | <0.10 ± 0.00 a | <0.10 ± 0.00 a  | <0.10 ± 0.00 a | <0.10 ± 0.00 a | <0.10 ± 0.00 a | <0.10 ± 0.00 a | <0.10 ± 0.00 a   | 0.50 ± 0.11 a  | <0.10 ± 0.00 a | <0.10 ± 0.00 a    | <0.10 ± 0.00 a | <0.10 ± 0.00 a  | %     |
| Peonidin-3-cumarylglucoside | <0.10 ± 0.00 a | <0.10 ± 0.00 a  | <0.10 ± 0.00 a | <0.10 ± 0.00 a | <0.10 ± 0.00 a | <0.10 ± 0.00 a | <0.10 ± 0.00 a   | <0.10 ± 0.00 a | <0.10 ± 0.00 a | <0.10 ± 0.00 a    | <0.10 ± 0.00 a | <0.10 ± 0.00 a  | %     |
| Peonidin-3-glucoside        | 18.9 ± 1.45 a  | 17.3 ± 1.77 a   | 15.0 ± 1.68 a  | 12.0 ± 2.92 a  | 16.4 ± 2.84 a  | 14.8 ± 2.55 a  | 18.5 ± 2.03 a    | 18.3 ± 2.12 a  | 18.1 ± 2.36 a  | 16.6 ± 2.81 a     | 17.0 ± 2.27 a  | 19.6 ± 2.10 a   | %     |
| Petunidin-3-glucoside       | 13.7 ± 2.36 a  | 14.8 ± 3.46 a   | 16.5 ± 2.12 a  | 18.7 ± 2.23 a  | 16.0 ± 2.74 a  | 16.9 ± 3.03 a  | 15.2 ± 2.59 a    | 15.1 ± 2.28 a  | 15.5 ± 2.15 a  | 16.7 ± 2.30 a     | 16.3 ± 2.51 a  | 13.7 ± 2.42 a   | %     |
| Caffeic acid                | n.d. ± 0.00 a  | n.d. ± 0.00 a   | n.d. ± 0.00 a  | n.d. ± 0.00 a  | n.d. ± 0.00 a  | n.d. ± 0.00 a  | n.d. ± 0.00 a    | n.d. ± 0.00 a  | n.d. ± 0.00 a  | n.d. ± 0.00 a     | n.d. ± 0.00 a  | n.d. ± 0.00 a   | mg/kg |
| Coumaric acid               | n.d. ± 0.00 a  | n.d. ± 0.00 a   | n.d. ± 0.00 a  | n.d. ± 0.00 a  | n.d. ± 0.00 a  | n.d. ± 0.00 a  | n.d. ± 0.00 a    | n.d. ± 0.00 a  | n.d. ± 0.00 a  | n.d. ± 0.00 a     | n.d. ± 0.00 a  | n.d. ± 0.00 a   | mg/kg |
| Ferulic acid                | n.d. ± 0.00 a  | n.d. ± 0.00 a   | n.d. ± 0.00 a  | n.d. ± 0.00 a  | n.d. ± 0.00 a  | n.d. ± 0.00 a  | n.d. ± 0.00 a    | n.d. ± 0.00 a  | n.d. ± 0.00 a  | n.d. ± 0.00 a     | n.d. ± 0.00 a  | n.d. ± 0.00 a   | mg/kg |
| Galic acid                  | 3.70 ± 0.88 a  | 2.73 ± 0.98 a   | 2.76 ± 0.83 a  | 3.07 ± 0.63 a  | 1.75 ± 0.99 a  | 1.60 ± 0.87 a  | 1.26 ± 0.28 a    | 1.54 ± 1.01 a  | 1.88 ± 0.87 a  | 1.57 ± 1.13 a     | 1.72 ± 0.89 a  | 2.38 ± 1.05 a   | mg/kg |
| Quercetin-3-O-glucoside     | 58.0 ± 4.55 a  | 53.3 ± 4.21 a   | 41.9 ± 4.90 b  | 48.8 ± 4.85 b  | 48.1 ± 5.12 b  | 67.3 ± 6.21 a  | 31.1 ± 3.71 c    | 48.7 ± 6.18 b  | 74.5 ± 9.12 a  | 35.3 ± 3.20 b     | 95.6 ± 19.54 a | 100.2 ± 22.88 a | mg/kg |
| Quercetin-3-O-galactoside   | 12.0 ± 2.51 a  | 10.8 ± 3.11 a   | 7.31 ± 2.08 a  | 8.91 ± 2.43 a  | 8.62 ± 2.58 a  | 13.1 ± 3.63 a  | 4.29 ± 3.10 b    | 10.2 ± 3.03 ab | 15.2 ± 3.62 a  | 6.43 ± 1.23 b     | 10.5 ± 2.87 b  | 24.2 ± 4.65 a   | mg/kg |
| Quercetin-3-O-glucuronide   | 70.4 ± 8.35 c  | 106.9 ± 17.11 a | 87.0 ± 16.42 b | 85.7 ± 9.55 a  | 73.2 ± 8.32 b  | 73.7 ± 8.38 b  | 30.9 ± 6.12 c    | 51.6 ± 6.14 b  | 69.0 ± 7.12 a  | 43.1 ± 5.60 c     | 79.5 ± 8.32 b  | 88.7 ± 7.47 a   | mg/kg |
| Quercetin-3-O-rutinoside    | 1.44 ± 1.07 a  | 3.72 ± 1.55 a   | 2.42 ± 1.23 a  | 2.50 ± 1.67 a  | 1.69 ± 1.32 a  | 1.04 ± 1.17 a  | 0.32 ± 0.25 a    | 0.63 ± 0.16 a  | 1.89 ± 0.34 a  | 1.31 ± 0.22 a     | 0.69 ± 0.27 a  | 1.45 ± 0.59 a   | mg/kg |
| Kaempferol-3-O-glucoside    | 7.04 ± 1.87 a  | 6.09 ± 1.57 a   | 4.61 ± 1.68 a  | 5.71 ± 1.29 a  | 5.04 ± 1.12 a  | 7.65 ± 1.99 a  | 1.49 ± 1.01 b    | 5.53 ± 1.75 ab | 9.31 ± 2.02 a  | 14.1 ± 2.54 a     | 5.66 ± 3.78 b  | 3.25 ± 1.15 b   | mg/kg |

Fractionation of anthocyanins (cyanidin-3-glucoside, delphinidin-3-glucoside, malvidin-3-acetylglucoside, malvidin-3-cumarylglucoside, malvidin-3-glucoside, peonidin-3-acetylglucoside, peonidin-3-cumarylglucoside, peonidin-3-glucoside and petunidin-3-glucoside) and coumaric acid, gallic acid, caffeic acid, ferulic acid, kaempferol-3-O-glucoside, quercetin-3-O-glucoside, quercetin-3-O-rutinoside, quercetin-3-O-galactoside and quercetin-3-O-glucuronide of *Vitis vinifera* treated with Zeowine, compost and control during the 2021 season. Measurements were conducted four times: full veraison (17 August 2021), mid maturation (30 August 2021), full maturation (9 September 2021) and harvest (13 September 2021). Data (mean ± s.e.,  $n = 5$ ) were subjected to one-way ANOVA. Different letters indicate significant differences among Zeowine, compost and control (LSD test,  $P \leq 0.05$ ).

**Table 4.** Phenolic maturity

|                             | 18 August 2022  |                 |                 | 28 August 2022  |                 |                 | 10 September 2022 |                 |                 | 15 September 2022 |                 |                 | m.u.  |
|-----------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-------------------|-----------------|-----------------|-------------------|-----------------|-----------------|-------|
|                             | Zeowine         | Compost         | Control         | Zeowine         | Compost         | Control         | Zeowine           | Compost         | Control         | Zeowine           | Compost         | Control         |       |
| Cyanidin-3-glucoside        | 18.8 ± 4.87 a   | 23.4 ± 4.18 a   | 14.8 ± 4.21 b   | 19.4 ± 5.23 a   | 19.5 ± 5.81 a   | 16.3 ± 4.07 a   | 22.0 ± 4.23 a     | 17.3 ± 2.23 ab  | 14.4 ± 3.28 b   | 17.4 ± 2.18 a     | 18.6 ± 3.63 a   | 19.7 ± 3.43 a   | %     |
| Delphinidin-3-glucoside     | 17.1 ± 3.76 a   | 17.3 ± 3.82 a   | 17.1 ± 3.54 a   | 17.6 ± 2.87 a   | 17.8 ± 2.45 a   | 17.7 ± 2.83 a   | 14.7 ± 3.56 a     | 15.0 ± 2.45 a   | 18.2 ± 3.12 a   | 13.3 ± 3.74 a     | 16.4 ± 2.21 a   | 15.4 ± 2.66 a   | %     |
| Malvidin-3-acetylglucoside  | <0.10 ± 0.00 a  | <0.10 ± 0.00 a  | <0.10 ± 0.00 a  | 0.50 ± 0.00 a   | 0.40 ± 0.00 a   | 0.50 ± 0.00 a   | <0.10 ± 0.00 a    | 0.50 ± 0.09 a   | <0.10 ± 0.00 a  | <0.10 ± 0.00 a    | <0.10 ± 0.00 a  | 0.40 ± 0.02 a   | %     |
| Malvidin-3-cumarylglucoside | <0.10 ± 0.00 a  | <0.10 ± 0.00 a  | <0.10 ± 0.00 a  | 0.50 ± 0.02 a   | 0.50 ± 0.02 a   | 0.50 ± 0.03 a   | 0.40 ± 0.04 a     | 0.60 ± 0.03 a   | 0.40 ± 0.02 a   | 0.70 ± 0.04 a     | 0.40 ± 0.03 a   | 0.50 ± 0.01 a   | %     |
| Malvidin-3-glucoside        | 33.6 ± 6.28 ab  | 29.0 ± 5.34 b   | 38.2 ± 6.14 a   | 32.1 ± 4.23 a   | 31.8 ± 5.98 a   | 35.1 ± 5.21 a   | 31.2 ± 4.87 a     | 36.4 ± 4.21 a   | 31.6 ± 5.32 a   | 37.2 ± 3.37 a     | 33.3 ± 4.23 a   | 32.5 ± 4.67 a   | %     |
| Peonidin-3-acetylglucoside  | <0.10 ± 0.00 a  | <0.10 ± 0.00 a  | <0.10 ± 0.00 a  | <0.10 ± 0.00 a  | <0.10 ± 0.00 a  | <0.10 ± 0.00 a  | <0.10 ± 0.00 a    | <0.10 ± 0.00 a  | <0.10 ± 0.00 a  | <0.10 ± 0.00 a    | <0.10 ± 0.00 a  | <0.10 ± 0.00 a  | %     |
| Peonidin-3-cumarylglucoside | <0.10 ± 0.00 a  | <0.10 ± 0.00 a  | <0.10 ± 0.10 a  | <0.10 ± 0.00 a  | <0.10 ± 0.00 a  | <0.10 ± 0.00 a  | <0.10 ± 0.00 a    | <0.10 ± 0.00 a  | <0.10 ± 0.00 a  | 0.40 ± 0.05 a     | <0.10 ± 0.00 a  | 0.40 ± 0.02 a   | %     |
| Peonidin-3-glucoside        | 12.3 ± 1.78 a   | 12.2 ± 1.98 a   | 9.90 ± 1.81 a   | 11.4 ± 1.33 a   | 11.5 ± 1.54 a   | 10.7 ± 2.01 a   | 15.3 ± 2.24 a     | 12.7 ± 2.44 a   | 11.2 ± 2.17 a   | 14.9 ± 2.87 a     | 13.0 ± 2.16 a   | 13.7 ± 2.52 a   | %     |
| Petunidin-3-glucoside       | 18.2 ± 2.81 a   | 17.6 ± 2.57 a   | 20.0 ± 3.04 a   | 18.6 ± 4.10 a   | 18.6 ± 4.71 a   | 19.1 ± 3.07 a   | 16.5 ± 2.86 a     | 17.5 ± 2.24 a   | 19.2 ± 2.67 a   | 16.0 ± 2.76 a     | 18.3 ± 2.71 a   | 17.3 ± 2.87 a   | %     |
| Caffeic acid                | <0.05 ± 0.00 a  | <0.05 ± 0.00 a  | <0.05 ± 0.00 a  | <0.05 ± 0.00 a  | <0.05 ± 0.00 a  | <0.05 ± 0.00 a  | <0.05 ± 0.00 a    | n.d. ± 0.00 a   | <0.05 ± 0.00 a  | <0.05 ± 0.00 a    | <0.05 ± 0.00 a  | <0.05 ± 0.00 a  | mg/kg |
| Coumaric acid               | <0.05 ± 0.00 a  | <0.05 ± 0.00 a  | <0.05 ± 0.00 a  | <0.05 ± 0.00 a  | <0.05 ± 0.00 a  | <0.05 ± 0.00 a  | <0.05 ± 0.00 a    | <0.05 ± 0.00 a  | <0.05 ± 0.00 a  | <0.05 ± 0.00 a    | <0.05 ± 0.00 a  | <0.05 ± 0.00 a  | mg/kg |
| Ferulic acid                | <0.05 ± 0.00 a  | <0.05 ± 0.00 a  | <0.05 ± 0.00 a  | <0.05 ± 0.00 a  | <0.05 ± 0.00 a  | <0.05 ± 0.00 a  | <0.05 ± 0.00 a    | <0.05 ± 0.00 a  | <0.05 ± 0.00 a  | <0.05 ± 0.00 a    | <0.05 ± 0.00 a  | <0.05 ± 0.00 a  | mg/kg |
| Gallic acid                 | 9.54 ± 2.27 ab  | 7.53 ± 1.14 b   | 15.2 ± 1.74 a   | 18.0 ± 2.20 a   | 8.9 ± 1.02 b    | 15.2 ± 3.44 ab  | 18.1 ± 2.63 a     | 21.3 ± 3.55 a   | 19.6 ± 2.19 a   | 16.5 ± 1.87 b     | 21.2 ± 3.87 a   | 11.3 ± 2.37 b   | mg/kg |
| Quercetin-3-O-glucoside     | 89.8 ± 11.37 b  | 126.2 ± 27.86 a | 72.4 ± 19.28 c  | 105.1 ± 35.17 b | 129.8 ± 20.78 a | 130.9 ± 23.68 a | 157.9 ± 18.78 b   | 170.3 ± 21.75 a | 179.0 ± 27.81 a | 118.1 ± 20.57 c   | 164.1 ± 21.78 b | 175.8 ± 24.27 a | mg/kg |
| Quercetin-3-O-galactoside   | <0.05 ± 0.00 a  | <0.05 ± 0.00 a  | <0.05 ± 0.00 a  | <0.05 ± 0.00 a  | <0.05 ± 0.00 a  | <0.05 ± 0.00 a  | <0.05 ± 0.00 a    | <0.05 ± 0.00 a  | <0.05 ± 0.00 a  | <0.05 ± 0.00 a    | <0.05 ± 0.00 a  | <0.05 ± 0.00 a  | mg/kg |
| Quercetin-3-O-glucuronide   | 178.3 ± 27.24 b | 231.4 ± 20.37 a | 134.2 ± 14.66 c | 183.5 ± 16.42 b | 172.4 ± 17.32 c | 215.6 ± 28.74 a | 163.5 ± 17.47 b   | 167.4 ± 16.54 b | 211.6 ± 26.30 a | 100.2 ± 14.57 c   | 165.9 ± 18.13 a | 151.8 ± 17.58 b | mg/kg |
| Quercetin-3-O-rutinoside    | 11.2 ± 1.53 a   | 7.63 ± 1.87 b   | 6.90 ± 1.93 b   | 9.32 ± 1.73 b   | 8.96 ± 1.76 b   | 12.3 ± 1.88 a   | 2.29 ± 0.34 b     | 9.02 ± 1.23 a   | 10.5 ± 2.67 a   | 2.18 ± 0.23 b     | 2.76 ± 1.01 b   | 7.59 ± 2.15 a   | mg/kg |
| Kaempferol-3-O-glucoside    | 26.1 ± 4.42 ab  | 32.1 ± 3.25 a   | 20.8 ± 2.17 b   | 34.2 ± 4.80 a   | 28.3 ± 2.55 b   | 30.8 ± 4.04 ab  | 35.4 ± 2.28 b     | 33.3 ± 3.21 b   | 46.8 ± 4.27 a   | 21.6 ± 3.11 b     | 34.4 ± 3.76 a   | 30.7 ± 3.27 a   | mg/kg |

Fractionation of anthocyanins (cyanidin-3-glucoside, delphinidin-3-glucoside, malvidin-3-acetylglucoside, malvidin-3-cumarylglucoside, malvidin-3-glucoside, peonidin-3-acetylglucoside, peonidin-3-cumarylglucoside, peonidin-3-glucoside and petunidin-3-glucoside) and coumaric acid, gallic acid, caffeic acid, ferulic acid, kaempferol-3-O-glucoside, quercetin-3-O-glucoside, quercetin-3-O-rutinoside, quercetin-3-O-galactoside and quercetin-3-O-glucuronide of *Vitis vinifera* treated with Zeowine, compost and control during the 2022 season. Measurements were conducted four times: full veraison (17 August 2022), mid maturation (26 August 2022), full maturation (10 September 2022) and harvest (19 September 2022). Data (mean ± s.e.,  $n = 5$ ) were subjected to one-way ANOVA. Different letters indicate significant differences among Zeowine, compost and control (LSD test,  $P \leq 0.05$ ).



**Figure 7.** Production. Cluster weight, yield per vine and number of cluster per vine of *Vitis vinifera* treated with Zeowine, compost and control during the 2021–2022 seasons. Measurements were conducted at harvest (13 September 2021 and 19 September 2022). Data (mean  $\pm$  s.e.,  $n=5$ ) were subjected to one-way ANOVA. Different letters indicate significant differences among Zeowine, compost and control (LSD test,  $P \leq 0.05$ ).

a more efficient way than the others, showing a lower leaf temperature and less negative water potential. In addition, we hypothesize that the zeolite addition to the soil made the roots

more humid in order to protect the leaves from photo-oxidative impairment (Wu *et al.*, 2019).

As reported in many studies, technological maturity was influenced by water and temperature stress (Zufferey *et al.*, 2017; Salvi *et al.*, 2020; Cataldo *et al.*, 2023). The compost and control treatments reported a more protracted ripening than Zeowine; in fact, a lower sugar content and a lower berry weight were found. According to Wang *et al.* (2003) water deficiency hampers sugar unloading in the berries. Conversely, Zeowine grapevines demonstrated a greater berry weight and higher production with increased cluster weight, confirming the positive impact of these particular aluminosilicates of alkaline and alkaline earth element on yield (Hazrati *et al.*, 2017). Absorption and controlled release of moisture by zeolite improves plant growth and yield under drought conditions (AL-Busaidi *et al.*, 2011). It has also been noted that zeolites can be effectively used in agriculture to encourage water infiltration and retention in the soil due to their porous and capillary skills that act as a slow-release source for water as well as macro or microelements (Rastogi *et al.*, 2019). Moreover, it was found that zeolite is capable of slowing urea release and, therefore, has the potential to be developed as controlling agent for the release of nitrogen from urea (Hidayat *et al.*, 2018). This considered, in addition to improving the administration of water resources, it was suggested that Zeowine increased the substrate cation exchange capacity which made nutrients available in the compost gradually and avoided losses due to leaching, affecting the final production (Confalonieri *et al.*, 2021; Zijun *et al.*, 2021). The pH parameter, however, was not influenced by treatment.

Regarding the anthocyanins, a general reduction in the content of totals and extractables was observed in all treatments in the 2022 season; this could be attributed to the scorching temperatures recorded in the moment of greatest synthesis (beginning of veraison – 1 August 2022, 35.1°C was enrolled) (Pirie and Mullins, 1977).

In extractable anthocyanins at the time of the two harvests, the following increases were recorded for treatment with Zeowine compared to compost and control, respectively: +7.68 and +7.87% on 13 September 2021; +24.5 and +20.5% on 13 September 2022. The increase in anthocyanins in the treatment with zeolites could in both years be attributed to the beneficial effect of this amendment on ripening. In fact, the treatment allowed a balanced and protected maturation from severe water deficit and intense thermal stress avoiding irreparable damage to the photosystem II (Shamili *et al.*, 2020) for correct storage of soluble solids. Furthermore, by improving the performance of the canopy, it improved thermal conditions by avoiding excessive increases in leaf temperature (probably verifiable also at cluster level).

Of the flavonols measured, as 3,5,7-trihydroxylated derivatives (hydroxylated in C3, C5 and C7), the glycosides from the following aglycons were identified: quercetin (3',4'-diOH) and kaempferol (4'-OH) (Castillo-Muñoz *et al.*, 2007), whereas the corresponding 3-O-galactoside derivatives were found to be minor compounds (Garrido and Borges, 2013). The highest concentrations of quercetin in grapes were found in the compost and control treatments. Quercetin-3-O-glucoside and kaempferol-3-O-glucoside have been found to be UV-B stress indicators in *V. vinifera* leaves in Pinot Noir and Riesling cultivars (Schoedl *et al.*, 2013; Boudierias *et al.*, 2020).

Therefore, it is believed that further research should be conducted, particularly to investigate the activity of anthocyanins

accumulated in the berry and the activity of phenylalanine as a precursor of various phenolic compounds.

## Conclusions

In order to decrease the environmental impact of agriculture, the efficiency of fertilizer use and water use must be maximized. The use of zeolite is key to improving plant water holding capacity which minimizes the requirement for vine irrigation, as water is well retained within zeolite's structure. This experiment supplies new evidence that zeolite applications could impact both physiological profiles and berry skin metabolism (sugar and size) of vines, providing an improved ability to counteract low water availability during the season. These findings support the need for consequent further investigations to be carried out. Primarily, research activities should consider the sugars accumulated in the vacuole (activity of sucrose-metabolizing enzymes, sucrose transporters and monosaccharide transporters).

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