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Effects of New Synthetic and Organic Hydrogel Soil Application on Physiology, Fruit Composition, and Wine Quality of Water-Stressed Potted Grapevines

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ABSTRACT

Background and Aims: Climate change poses increasing issues to the rapid and successful establishment of new vineyards. In this study, conducted over two consecutive seasons on potted vines, we evaluated the effects of a synthetic potassium polyacrylate hydrogel (WS-SH) and an organic-based hydrogel (WS-OH), both applied at transplanting, on vine water status, productivity, and fruit and wine composition, under progressive water deficit. These treatments were compared with a water-stressed control (WS-C) and a fully irrigated reference (WW-C).

Methods and Results: In both years, WS-SH and WS-OH treatments delayed the decline in predawn water potential by several days and improved leaf gas exchange under limited irrigation. This led to increased leaf area and overall vine vigor. By the end of the second year, WS-OH had greater root system development compared to all other water-stressed treatments (+17% than WS-C), while WS-SH favored dry matter allocation toward aboveground organs (+17.6%). As a result, both WS-SH and WS-OH vines maintained yields comparable to WW-C, whereas WS-C showed a 33% yield reduction. Due to higher crop loads, WS-SH and WS-OH vines had lower leaf-to-fruit ratios than WS-C ($-0.116 \text{ m}^2/\text{kg}$ and $-0.147 \text{ m}^2/\text{kg}$, respectively), resulting in reduced grape sugar content (-1.8 and -1.6°Brix , respectively), anthocyanins (-48% and -51% , respectively), and phenolics (-21% and -23% , respectively). Wines produced at the end of the second season reflected this composition, showing significantly lower alcohol content (-1.11% v/v and -1.05% v/v, respectively), phenolic levels, and chromatic traits.

Conclusions: Our results demonstrate that hydrogels applied at transplanting can enhance vine tolerance to water stress and increase yield in the first productive season, in exchange for a reduction in sugar and anthocyanin contents in grapes.

Significance of the Study: Hydrogels could represent valuable tools to increase vineyard tolerance to summer stresses after transplanting. No substantial differences were observed between the effects of the synthetic and organic hydrogels, highlighting the potential of organic formulations as a sustainable and effective strategy to improve vineyard resilience to summer drought conditions. These results should be confirmed under operational conditions through field experiments.

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1 | Introduction

Hydrogels are innovative materials rapidly gaining attention among agriculture specialists due to their unique properties and the increasing pressures posed by climate change [1–3]. They are defined as materials capable of absorbing substantial amounts of water relative to their mass, owing to their specific biochemical configuration, consisting of long-chain polymers rich in hydrophilic reactive groups [4, 5]. Recent studies have shown a rapid expansion in their use, with a growing variety of new formulations becoming available on the market, showing a higher efficacy in terms of water absorption and soil trait improvement than the traditional ones [6, 7]. The water absorption capacity of hydrogels varies widely, ranging from approximately 8–400 g of water per g of hydrogel, depending on their formulation and origin, but many traditional and new formulations have proven especially effective in supplying water to plants under severe drought conditions [1, 8, 9]. Notably, natural, organic, bio-based hydrogels are today playing a transformative role by eliminating reliance on fossil-derived precursors and preventing potential microplastic contamination of soils, issues commonly associated with polyacrylate-based hydrogels, even if the degradation pattern of both synthetic and natural hydrogels needs further specific evaluations [5, 6, 10]. Natural hydrogels are derived from lignocellulosic or carbohydrate-based materials and typically exhibit a lower, yet still effective, water absorption capacity [5, 11].

While the scientific literature contains numerous studies and review papers on the effects of conventional hydrogels on plant survival under water stress or drought conditions [12, 13], significantly fewer studies have examined next-generation hydrogels. Even fewer focus on tree crops [14–17], and only a handful specifically address their application in viticulture [18–20]. In general, for grapevines and tree crops, the most effective use of hydrogels is through localized soil application at transplanting. This approach aims to reduce plant mortality, enhance tolerance to water stress despite small root systems, accelerate the development of permanent structures, and promote earlier achievement of full cropping conditions [14, 21]. In previous work, we assessed the chemical and physical properties of three different hydrogel types, aiming to determine their potential to enhance the field capacity and maximum available water of a sandy loam–clay soil, in relation to vine requirements. The results demonstrated that localized application of both polyacrylate and natural hydrogels significantly improved key soil hydrological properties, providing additional water per gram of soil at water potential levels suitable for vine root uptake [19]. With regard to polyacrylate-based hydrogels, soil incorporation at transplanting is capable of increasing fertilization efficiency, chlorophyll accumulation, and photosynthetic efficiency in the absence of limiting conditions [18, 20]. In a trial conducted under controlled conditions, a synthetic hydrogel postponed the decrease of soil water potential and improved photosynthesis at comparable stress levels, improving assimilation gains at rewetting [18]. In field conditions, only preliminary studies have been conducted, and while positive effects in water-stressed woody plants and vines physiology have been proved [15–17, 20, 22], the efficacy of hydrogels in improving vine survival at transplanting, reducing duration of unproductive stages, and increasing yield still needs to be demonstrated.

Specifically, the literature lacks studies assessing the use of hydrogels for sustaining productivity and fruit quality during the

initial cropping seasons. On other tree crops, some studies have reported the effects of hydrogel application on fruit composition, but only when applied to mature productive trees. In irrigated 24-year-old olive trees, the use of synthetic hydrogels increased olive oil yield by 79%, without compromising oil quality [15]. Similarly, in six-year-old mango trees, synthetic hydrogels led to progressive increases in both yield and fruit sugar content with higher application rates [23]. In grapevines, the only available study investigated a combined application of synthetic hydrogel and nitrogen fertilizers on irrigated, seven-year-old vines, resulting in an 8.3% yield increase and a 0.8°Brix rise in total soluble solids (TSSs), compared to the nonfertilized control [20]. However, there is a complete lack of research on the effects of hydrogel application during transplanting on fruit quality. Moreover, no studies to date have evaluated the performance of nonsynthetic, organic-based hydrogels on fruit quality, grape composition, and final wines.

Therefore, the aim of this study was to evaluate the effects of soil incorporation at transplanting of a new-generation synthetic hydrogel and a lignin-sulfonate-based organic hydrogel on the productive performance of young potted grapevines and the composition of the resulting grapes and wines. Under semi-controlled conditions, we were able to impose a progressive water stress during the hottest summer days over two seasons, reaching stomatal closure, followed by rewetting until harvest. The overall objective was to determine whether these two hydrogels could enhance vine physiology and yield by the second year after transplanting, and if this affects fruit composition and wine biochemical characteristics.

2 | Materials and Methods

2.1 | Plant Material, Treatment Layout, and Experimental Setup

The study was conducted in 2023 and 2024, on 16 one-year-old vines cv. Sangiovese clone VCR5 grafted SO4 rootstock, in an outdoor area of Università Cattolica del Sacro Cuore, in Piacenza, Italy (44°55' N, 9°44' E). All the plants were potted on March 23, 2023, in a uniform substrate made of a blend of river sand (70%) and loamy soil (30%). Final texture was sand 54%, clay 27%, and silt 19%; organic matter concentration was 0.8%; and cation exchange capacity was 19.3 meq/100 g. Right before transplanting, the 16 vines were assigned to the four following treatments (four vines per treatment): (i). well-watered vines with no hydrogel application (well-watered control [WW-C]); (ii). water-stressed vines with no hydrogel application (WS-C); (iii). water-stressed vines with incorporation of a synthetic potassium polyacrylate-based hydrogel into the soil (WS-SH), (iv). water-stressed vines with incorporation of a lignin-sulfonate-based hydrogel (permitted in organic agriculture) into the soil (WS-OH). In WS-SH and WS-OH vines, the hydrogels were applied by uniformly dispersing the dry granules in the substrate at transplanting on March 23, 2023, avoiding the bottom 5 cm from the base of the pot and the portion of soil above the first root ramification.

Due to the different maximum water absorbed by the two hydrogels [19], dosages were 30 g/vine for WS-SH and 100 g/vine for WS-OH. In both treatments, the doses were tuned for a maximum absorption by the sole hydrogels of 2.5 L/0.05 m³ substrate and 2.5 L/vine, in agreement with [19]. All pots were

fertilized with 3.25 g of N at transplanting by applying Multicote 13-5-21, a granular fertilizer distributed in the soil in the same way as the hydrogels. Additionally, during the season, three applications of 1.25 g of N from ammonium nitrate (27%) were made on April 20, 2023; May 02, 2023; and May 11, 2023. In total, 7 g of N per vine was applied in 2023 through surface soil application before light irrigation. Each vine was standardized in 2023, retaining the two best shoots developing and removing the others. In 2024, all vines were pruned retaining the best cane and shortening it to 11 nodes. In spring 2024, shoot thinning was executed at BBCH 15 [24], retaining 8 shoots per vine. Vines were arranged on a vertically shoot-positioned trellis along a single row having a 35° NE–SW orientation. Three upper foliage wires were positioned above the vine's edge for a canopy wall that extended above the graft-union by about 1.8 m. Shoots were trimmed when they exceeded 20 cm above the last wire.

2.2 | Irrigation Management

Irrigation was managed using an automated system that supplied water daily at 8:00 a.m. For all the vines, weekly adjustments were made to ensure daily application of 100% of the 24-h evapotranspiration (ET), maintaining the soil at field capacity each morning. The 100% ET value was determined gravimetrically by measuring the weight of three WW-C vines immediately after full irrigation and again 24 h later. In both years, a progressive reduction in irrigation was implemented for WS-C, WS-SH, and WS-OH vines during periods of high temperature, continuing until stomatal closure was observed. After this point, full irrigation was resumed for the rest of the season. Specifically, in 2023, irrigation was reduced to 50% ET starting on July 29 (day of year [DOY] 210), and from August 3 (DOY 215), irrigation was completely suspended until August 6 (DOY 218), when stomatal closure was achieved. In 2024, irrigation was reduced to 50% ET on June 20 (DOY 171), when vines were at pea-size berries phenological stage (BBCH 75) [24] and fully suspended on June 29 (DOY 180) until July 2 (DOY 183), at mid-veraison (BBCH 83).

2.3 | Vine Water Status, Leaf Gas Exchange, Fluorescence, and Metabolites

In both years, starting one day before water stress imposition, predawn water potential (Ψ) was measured every 2-3 days on three vines per treatment to determine soil and vine water status. Measurements were taken between 3:30 a.m. and 4:30 a.m., with a Scholander pressure chamber.

Leaf gas exchanges were analyzed on August 4, 2023 (DOY 216), and July 1, 2024 (DOY 182), when WS-C predawn Ψ was approaching -0.9 MPa. Readings of leaf assimilation rates (A), transpiration rates (E), and stomatal conductance (g_s) were taken between 12:00 and 13:00 through a portable gas exchange infrared gas analyzer LCi-SD (ADC Bio Scientific Ltd., Hertz, UK), on mature leaves inserted on nodes 3–7 of main shoots (four leaves per treatment), under saturating light conditions. The system was equipped with a broad (6.25-cm²) cuvette chamber. All measurements were performed at ambient relative humidity with the adjustment of airflow at 200 mL min⁻¹. Water use efficiency (WUE) was calculated as the ratio of Leaf A and Leaf E.

Chlorophyll fluorescence was measured on the same dates of predawn Ψ measurement, at 12:00, on a mature well-exposed leaf per vine, using the field-portable pulse-modulated fluorimeter

Handy-PEA (Hansatech Instruments, Norfolk, UK). Segments of leaves were dark-adapted for a period of 45 min via the use of leaf-clips supplied with the instrument.

After fluorescence measurement, leaves were sampled and immediately frozen in liquid nitrogen, then stored at -80 °C. Leaves were then ground in the lab to a fine powder under liquid nitrogen. Leaf proline concentration was determined after [25]. 50 mg of ground leaves was mixed with 500 μ L of ethanol–water (70:30 v/v) and 1000 μ L of a mix of 1% ninhydrin (w/v) in acetic acid 60% (v/v), ethanol 20% (v/v), and water. The sample was heated at 95°C for 20 min, centrifuged at 10,000 rpm for 1 min. Then, absorbance was read at 520 nm on a JascoV-530 spectrophotometer (Jasco Analytical Instruments, Easton, MD, USA). Hydrogen peroxide (H₂O₂) was determined after [26]. 0.05 g of dry leaf was mixed with 1 mL 1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 10,000 rpm for 5 min at 4°C. Then, 0.75 mL of the supernatant was added to 0.75 mL of phosphate buffer (pH 7.0) and 1.5 mL of 1M potassium iodide solution. The absorbance was read at 390 nm.

Leaf nitrogen (N), phosphorus (P), and potassium (K) were analyzed on leaves sampled the same day of chlorophyll fluorescence analysis (four primary mature leaves per treatment, inserted at nodes 3–8). Leaf N and Leaf P were analyzed according to [27, 28] standards. K was quantified according to the methods described by [29].

2.4 | Leaf Area, Pruning Weight, Reserves, and Dry Matter (DM) Partitioning

Leaf area was estimated at the end of each season by counting, on a vine basis, the number of nodes developed on primary shoots and lateral shoots, and multiplying the total by the average size of leaves from primary and lateral shoots, respectively. The average size of leaves was obtained by sampling before leaf fall, one entire shoot per vine, and measuring on each sample the number of leaves from primary shoots and of leaves from lateral shoots, and total leaf area was measured with a leaf area meter LI-COR Li3100c (LI-COR Environmental, Lincoln, NE, USA). Pruning weight was measured by weighing the mass of 1-year-old wood at pruning. At the end of 2024, vines were destructively dismantled, and in addition to one-year-old wood, the fresh weight of leaves (detached before leaf fall), elder wood, and roots was measured on each vine. Subsamples of leaves, grapes (taken at harvest), one-year-old wood, elder wood, and roots per vine were collected, weighed, and then kept at 105 °C in a ventilated oven for 2 weeks. Dry weight was measured, and DM % was calculated. DM allocation and partitioning to aboveground organs were calculated by summing leaves, grapes, one-year-old wood, and elder wood DM allocation.

A sample of one-year-old wood from the third internode and of fine roots having a diameter of 5 mm was collected on each vine at pruning (end of 2023 season) and at vine dismantling (end of 2024 season), for the analysis of reserves. Alcohol-soluble sugars and starch in both organs were determined using anthrone reagent (Merck) as described by [30]. Absorbance was read at 620 nm with a Jasco V-630 spectrophotometer.

2.5 | Yield Components and Grape and Must Compositions

Commonly, grapes from pot-grown vines irrigated at field capacity during the harvest period do not reach sugar

concentrations comparable to those achieved under field conditions [31–33]. Therefore, vines were harvested when the grapes reached a TSS concentration of 18°Brix as an average of the treatments (September 3, 2024). The number of bunches per vine was recorded, and yield was measured on a per-vine basis using a portable scale. Yield in tons per hectare was then calculated based on a vine density of 3333 vines/ha, according to the average length of fruiting canes (1.2 m) and standard spacing between vineyard rows in VSP systems (2.5 m). The leaf-to-fruit ratio was determined by dividing the total leaf area per vine by the vine yield. The Ravaz Index was calculated as the ratio of vine yield to vine pruning weight. Three representative clusters per vine were sampled to measure the number of berries per bunch, berry mass, and rachis length, and to calculate bunch compactness (g/cm). A 100-g berry sample from each vine was stored for the analysis of total anthocyanins and phenolics in the grapes, as well as skin anthocyanidins and flavonols. The remaining grapes were crushed to obtain musts on which TSS, pH, titratable acidity (TA), and malic and tartaric acid concentrations were analyzed.

TSS concentration was measured using a digital refractometer SMART-1 (Atago, Bellevue, WA, USA), and pH was assessed with a pH meter (pH 60 VioLab Giorgio Bornac, Carpi, MO, Italy). TA was measured with an AT 1000 Potentiometer titrator (Hach Company, Loveland, CO, USA) by titrating 0.1N NaOH to a pH of 8.2 as the endpoint, expressed as g/L tartaric acid equivalents.

The concentration of malic and tartaric acids of grape juice was analyzed through an automatic enzymatic analyzer (Hyperlab Smart, Steroglass, San Martino in Campo, PG, Italy). Briefly, 50 mL of grape mash was centrifuged at 15,000 rpm for 5 min at 4 °C and the supernatants were immediately analyzed. Each sample was carried out in duplicate.

Total anthocyanins and phenolics in grapes were determined using a sample of 80 berries per vine, following the method described by [34]. Berries were homogenized at 24,000 rpm with an Ultra-Turrax T25 (Rose Scientific Ltd., Edmonton, Canada) homogenizer for 5 min; afterward, 2 g of the homogenate was put into a centrifuge tube, added with 10 mL of aqueous ethanol extraction solution (50%, pH 5), and kept for 1 h, mixing every 10 min. After the extraction period, the solution was centrifuged at 3500 rpm, and after 5 min, 0.5 mL supernatant was added to 10 mL 1M HCl. After three hours, absorbance was read at 520 nm for total anthocyanins and 280 nm for total phenolics, on a JascoV-530 spectrophotometer (Jasco Analytical Instruments, Easton, MD, USA). Total anthocyanin and phenolic concentrations were expressed as mg per g of fresh weight.

The remaining 20 berries per vine were carefully manually peeled, and the resulting skins were immediately frozen and lyophilized. Phenolic compounds were extracted from grape skins, following [35]. In brief, 0.1 g of lyophilized grape skin was extracted in 1.0 mL of 50% (v/v) methanol in water for 15 min with sonication. The extracts were centrifuged (5 min at 10,000 × g at 4°C), filtered through a 0.22-μm polypropylene syringe for HPLC analysis, and transferred to HPLC autosampler vials. A chromatographic method was developed using an Agilent 1260 Infinity Quaternary LC (Agilent Technology, Santa Clara, CA, USA), which consisted of a G1311 B/C quaternary pump with an inline degassing unit, a G1329B autosampler, a G1330B thermostat, a G1316B thermostated column

compartment, a G4212B diode array detector (DAD) fitted with a 10-mm path, and a 1 μL Max-Light cartridge flow cell. The instrument was controlled using the Agilent ChemStation software Version A.01.05. Separation was achieved on a reverse-phase C18 Synergi Hydro-RP 80A, 250 × 4.6 mm, 4 μm (Phenomenex manufacturers, Torrance, CA, USA), according to Nicoletti et al. [36]. The solvent used comprised 5% (v/v) formic acid (Solvent A) and acetonitrile (Solvent B). The flow rate was 0.5 mL·min⁻¹, with a linear gradient profile consisting of Solvent A with the following proportions (v/v) of Solvent B: 0–10 min, 2%–10% B; 10–25 min, 10%–12% B; 25–35 min, 12%–30% B; 35–43 min, 30% B; 43–48 min, 30%–40% B; 48–52 min, 40%–50% B; 52–55 min, 50%–60% B; 55–58 min, 60%–98% B; 58–63 min, 98% B; 63–66 min, 98%–2% B; and 66–72 min, 98% B. The column temperature was maintained at 40 ± 0.1°C, and 5 μL of the sample extract was injected. The elution was monitored at 200–700 nm, and the detection was done through UV–visual absorption with DAD scanning among 280, 320, 370, and 520 nm. Phenolic compounds were identified using authentic standards and by comparing the retention times. Quantification was based on peak areas and performed by external calibration with standards. All anthocyanins were thereby expressed as malvidin 3-O-glucoside.

2.6 | Winemaking and Wine Analysis

On September 3, 2024, after harvest, all the resulting grapes were brought to the experimental winery of Università Cattolica del Sacro Cuore. Each grape lot was manually destemmed, and three replicates of about 4 kg of berries per treatment were obtained and crushed in 5-L fermentation plastic vats (Polsinelli Enologia, Isola del Liri, FR, Italy). Musts were added with 40 mg/L of potassium metabisulfite. The grape mashes were inoculated with 30 g/hL of *Saccharomyces cerevisiae* (*Zymaflore FX10*, Laffort Italia s.r.l., Tortona, AL, Italy), and the pomace was manually punched down twice a day. The fermentations were carried out at 24 °C ± 2 °C. At the end of the alcoholic fermentation, the wines were racked off and added with 20 mg/L of potassium metabisulfite. Finally, the wines were bottled in 330-mL crown-capped dark glass bottles and stored at 12 °C for 2 months prior to analysis.

For the wine analysis, two bottles for each fermentation batch were used. Methanol, ethanol, 1-butanol, acetaldehyde, sodium metabisulfite, sodium nitrite, sodium molybdate, iron (II) sulfate heptahydrate, sulfuric acid, hydrochloric acid, malvidin, catechin, gallic acid, vanillin, and Folin–Ciocalteu reagent were purchased from Sigma-Aldrich (St. Louis, MO, USA). All the reagents had a purity level of ≥ 99%. The alcohol content was analyzed through an AlcoLyzer 5001 (Anton Paar Italia, Rivoli, TO, Italy), according to [37]. TA, pH, all chromatic properties, and Folin–Ciocalteu were determined according to OIV [38]. The organic acid profile (tartaric, malic, and citric acid) was determined according to [39] with a few modifications: Briefly, 3 mL of each wine was filtered on 0.22-μm membranes (Sartorius, Germany) and analyzed with a PerkinElmer Series 200 HPLC system (PerkinElmer, CT, USA) coupled with a UV detector set to 210 nm. The analyses were performed isocratically at 0.5 mL/min at room temperature using a Phenomenex Rezex ROA organic acid H⁺ (8%) column (300 mm x 7.8 mm), with 0.005 N H₂SO₄ as the mobile phase. All the spectrophotometric measurements were performed on a Jasco V-730 UV–Vis

spectrophotometer (Jasco Europe, Cremella, LC, Italy) using quartz 1/10-mm cuvettes. Total phenols, cinnamic acids, total flavonoids (TFs), total anthocyanins, proanthocyanidins, and flavans reactive with vanillin (FRV) were analyzed according to [40, 41]. The copigmented, monomer, and polymer anthocyanin fractions were determined according to [42].

2.7 | Statistical Analysis

The dataset was first analyzed to test the analysis of variance (ANOVA) assumptions of homoscedasticity and normal distribution. Data available over one season were then subjected to a one-way ANOVA with SPSS Statistics 24.0 (IBM, Armonk, NY, USA). Data available over 2 years were subjected to a two-way ANOVA (treatment, year) with the same software. Student–Newman–Keuls (SNK) post hoc comparison test was used to assess the statistical significance between treatments ($p < 0.05$).

3 | Results

3.1 | Vine Physiological Performances and Stress-Related Metabolite Leaf Accumulation

In 2023, when irrigation was reduced to 50%, all water-stressed vines experienced a slight decline in predawn Ψ . However, on DOY 216 (the first day of full irrigation suspension), WS-OH vines maintained a significantly higher Ψ compared to WS-C and WS-SH (Figure 1(a)). By DOY 218, predawn Ψ in WS-C had dropped to -0.84 MPa, while WS-OH and WS-SH recorded higher values of -0.45 MPa and -0.61 MPa, respectively. At the final measurement before rewatering (DOY 219), WS-C reached a predawn Ψ of -1.05 MPa, whereas WS-SH and WS-OH maintained significantly higher levels, averaging -0.84 MPa. In 2024, no differences in predawn Ψ were observed among water-stressed vines on DOY 180 (Figure 1(b)). However, by DOY 182, WS-OH maintained a significantly higher predawn Ψ than WS-C, with a difference of $+0.34$ MPa, while WS-SH showed intermediate values between the other two treatments.

In both years, WS-C vines exhibited a significant decline in leaf Fv/Fm compared to all other treatments by the end of the experiment, with reductions of 34% in 2023 and 17% in 2024 (Figures 1(c) and 1(d)). In 2023, WS-OH also showed a decrease in leaf Fv/Fm, although it was significantly different from WW-C only on DOY 219. WS-C vines accumulated substantially more leaf proline than any other treatment. On DOY 219, while WW-C leaves had 5.72 $\mu\text{mol/g}$, WS-C vines reached 23.84 $\mu\text{mol/g}$. WS-OH values were similar to WW-C, whereas WS-SH had an intermediate proline concentration of 11.75 $\mu\text{mol/g}$ (Figure 1(e)). In 2024, the increase in leaf proline in WS-C was lower than in the previous year. However, on DOY 182, WS-C still showed a 140% higher leaf proline concentration compared to WS-SH and WS-OH (Figure 1(f)).

Similarly, WS-C showed the highest leaf H_2O_2 accumulation among all treatments. On DOY 218 in 2023, WS-C leaves reached 1.108 $\mu\text{mol/g}$, compared to 0.567 $\mu\text{mol/g}$ in WS-OH and 0.517 $\mu\text{mol/g}$ in WS-SH (Figure 1(g)). A similar pattern was observed in 2024, with no significant differences in leaf H_2O_2 levels among WW-C, WS-SH, and WS-OH. In contrast, from DOY 218 onward,

WS-C exhibited 51% higher H_2O_2 concentrations compared to the average of the other treatments (Figure 1(h)).

In 2023, on DOY 216, while WS-C showed null photosynthesis and transpiration, WS-SH and WS-OH were displaying a Leaf A of 4.32 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 3.22 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively, while in WW-C, Leaf A was 9.84 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Figure 2(a)). A symmetric pattern was observed on DOY 182 of 2024 (Figure 2(b)). Differences between WS-C, WS-OH, and WS-SH in terms of Leaf E were much lower (Figures 2(c) and 2(d)), especially in 2023. As a result, WS-OH showed a significantly higher leaf WUE than WS-C in 2023 ($+7.61$ $\mu\text{mol m}^{-2} \text{s}^{-1}/\text{mmol m}^{-2} \text{s}^{-1}$) (Figure 2(e)) and 2024 ($+1.87$ $\mu\text{mol m}^{-2} \text{s}^{-1}/\text{mmol m}^{-2} \text{s}^{-1}$) (Figure 2(f)), and WS-SH only in 2024 ($+1.13$ $\mu\text{mol m}^{-2} \text{s}^{-1}/\text{mmol m}^{-2} \text{s}^{-1}$).

3.2 | Vegetative Growth, Leaf Nutrients, DM Partitioning, and Reserve Accumulation

Averaged across both seasons, WS-SH vines had a significantly greater main shoot leaf area and total vine leaf area compared to WS-C, with no differences observed in the contribution from lateral shoots (Table 1). However, Supporting Figure 1 shows that in 2023, WS-C had significantly lower leaf area and pruning weight compared to WW-C, whereas WS-SH and WS-OH did not exhibit such reductions in vegetative growth. In 2024, WS-SH again exhibited a significantly larger leaf area than WS-C in main shoots (Supporting Figure 1(a)), lateral shoots (Supporting Figure 1(b)), and total vine leaf area (Supporting Figure 1(c)). Notably, in 2024, both WW-C and WS-OH also showed greater lateral shoot leaf area and total vine leaf area than WS-C. A similar trend was observed in 2023 for pruning weight (Supporting Figure 1(d)).

WS-OH leaves had the highest leaf nitrogen concentration among all treatments (2.11% DM), while WS-SH also showed significantly higher Leaf N levels than WS-C. No significant differences among treatments were observed in Leaf P concentration. However, WS-SH displayed a higher K concentration compared to the other treatments.

By the end of 2024, WS-SH and WS-OH allocated significantly more DM to grapes and one-year-old wood than WS-C (Table 2). In contrast, WS-C allocated the least DM to roots (17% less than WS-OH). Consequently, WS-SH exhibited the highest above-ground-to-belowground DM allocation ratio.

While WS-SH and WS-OH vines had a comparable third internode diameter to WW-C vines, WS-C had a significantly reduced diameter of the third internode (-0.2 cm than other treatments pooled together) (Table 3). A significant interaction between treatment and year was observed for soluble sugars and starch concentrations in the third internode and roots. In 2023, the only statistically significant difference was the higher root starch concentration in WS-SH and WS-OH compared to WW-C and WS-C (Supporting Figure 2(d)). In 2024, no significant differences were found in root carbohydrate concentrations (Supporting Figure 2(c)); however, WS-SH had a lower soluble sugar concentration in the third internode compared to the other treatments (Supporting Figure 2(a)), while WW-C exhibited the lowest starch concentration in the same tissue (Supporting Figure 2(b)).

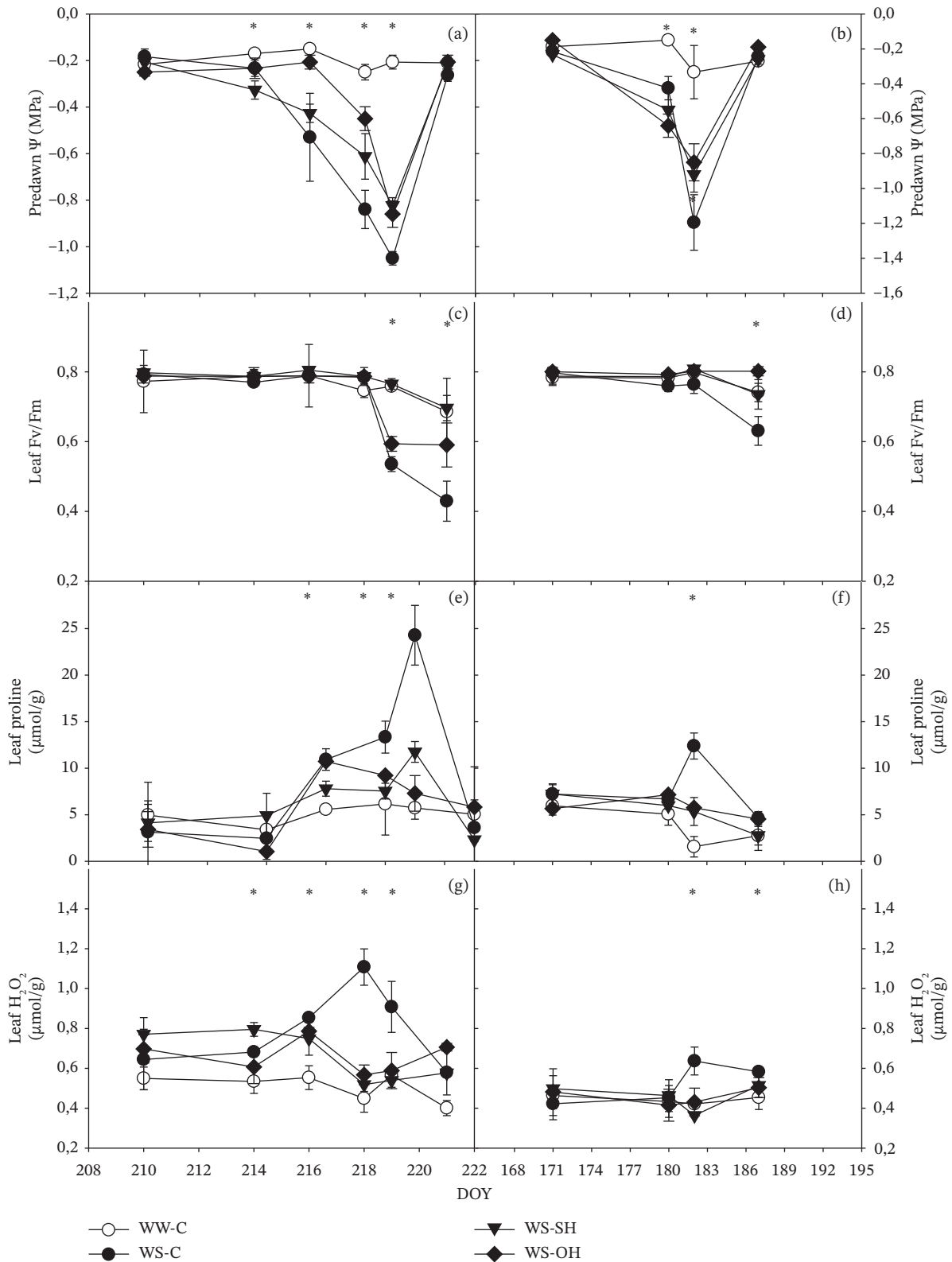


FIGURE 1 | Predawn water potential (Ψ , panels a and b), leaf Fv/Fm (panels c and d), leaf proline concentration (panels e and f), and leaf hydrogen peroxide (H_2O_2) concentration (panels g and h) in vines subjected to soil application of different hydrogels at transplanting, and in control vines, in 2023 (panels a, c, e, and g) and in 2024 (panels b, d, f, and h). Means \pm standard errors ($n = 3$). WW = well-watered; WS = water-stressed; C = control; SH = synthetic hydrogel; OH = organic hydrogel. Asterisks indicate within-date significant differences between treatments ($p < 0.05$).

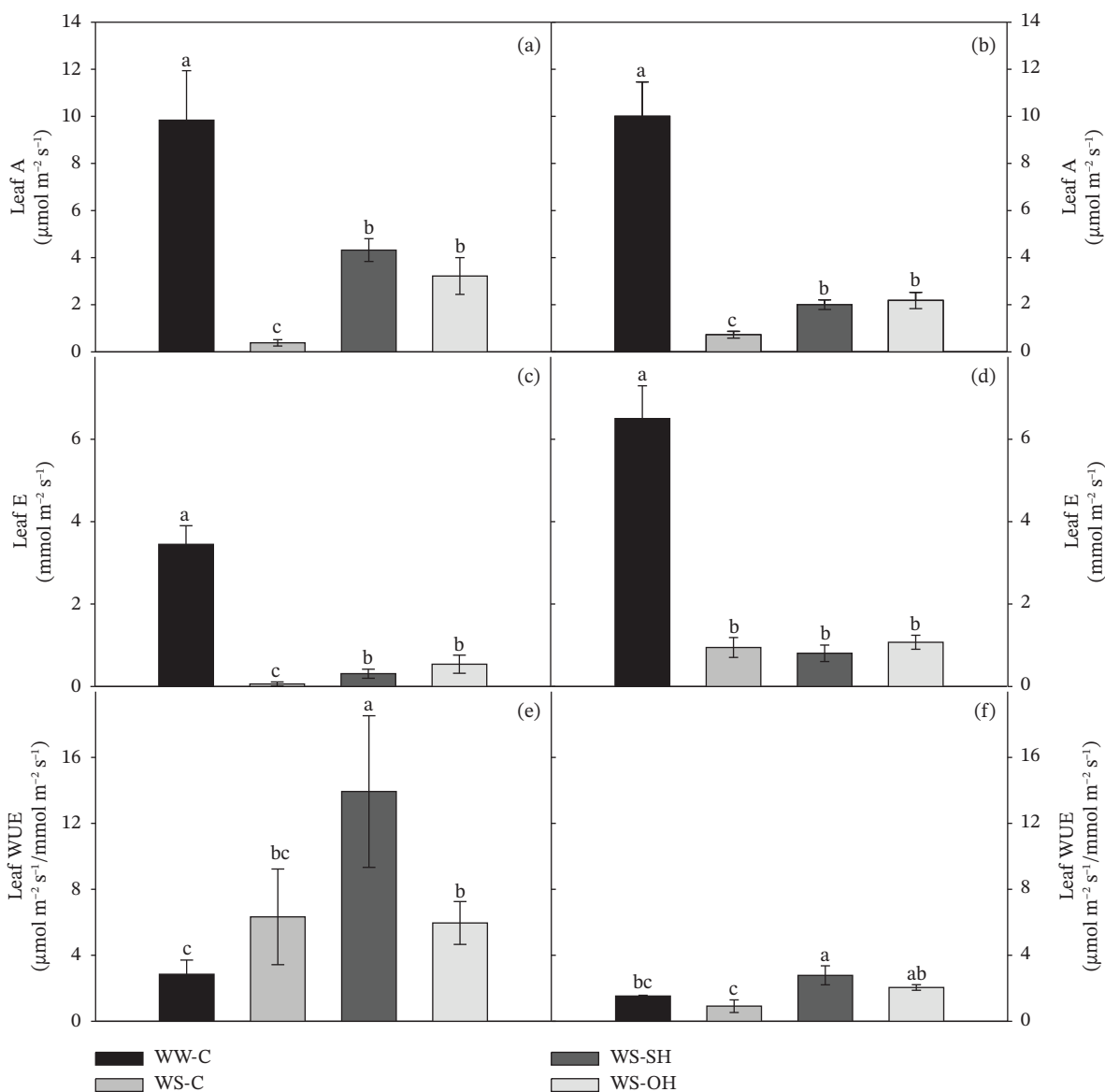


FIGURE 2 | Leaf photosynthesis (A, panels a and b), transpiration (E, panels c and d), and water use efficiency (WUE, panels e and f) in vines subjected to soil application of different hydrogels at transplanting, and in control vines, in August 4, 2023 (day of year[DOY] 216) (panels a, c, and e), and in 1 July 2024 (DOY 182) (panels b, d and f). Means \pm standard errors ($n = 3$). WW = well-watered; WS = water-stressed; C = control; SH = synthetic hydrogel; OH = organic hydrogel. Different letters within columns indicate significant differences per $p < 0.05$ (SNK test).

3.3 | Yield Components and Grape and Must Quality

In 2024, WS-C displayed a reduced yield as compared to WW-C (-33%), while WS-SH and WS-OH maintained a comparable yield to WW-C (Table 4). The reduction of yield was due to both the number of bunches per vine (-2 bunches/vine vs. the other treatments) and the average bunch weight (-16% as compared to WW-C). WW-C had a significantly higher berry mass than WS-C, WS-SH, and WS-OH, and no difference was found in terms of the number of berries per bunch. As a result, WS-C had a much higher leaf-to-fruit ratio than WW-C ($+0.157 \text{ m}^2/\text{kg}$). Both WS-SH and WS-OH had a significantly lower leaf-to-fruit ratio than WS-C ($-0.116 \text{ m}^2/\text{kg}$ and $-0.147 \text{ m}^2/\text{kg}$, respectively). In addition, WS-C had a significantly lower Ravaz Index than other treatments.

At harvest, grapes from the WW-C and WS-C treatments had significantly higher must TSS than WS-SH and WS-OH treatments (Table 5). WW-C also exhibited significantly higher TA than WS-SH and WS-OH, which in turn had higher TA than WS-C. Consequently, the TSS/TA ratio was notably higher in WS-C compared to the other treatments, while WW-C maintained the lowest ratio. WW-C showed the lowest malic acid concentration and the highest tartaric acid concentration. Both WS-SH and WS-OH had higher malate levels than WS-C, resulting in WW-C having the highest tartaric-to-malic acid ratio. Additionally, WS-C grapes had higher concentrations of anthocyanins and phenolics than any other treatment, with WS-SH and WS-OH showing the lowest contents.

Analysis of the anthocyanidin profile suggests that the lower chromatic potential of WS-SH and WS-OH is primarily due to

TABLE 1 | Final leaf area, pruning weight, and leaf nitrogen (N), phosphorus (P), and potassium (K) concentration in vines subjected to the soil application of different hydrogels at transplanting, and in control vines, in 2023 and 2024.

	Main shoots LA (m ² /vine)	Lateral shoots LA (m ² /vine)	Total vine LA (m ² /vine)	Pruning weight (g/vine)	Leaf N (%DM)	Leaf P (%)	Leaf K (%)
WW-C	1.00 ab ²	1.29	2.29 a	300 ab	1.82 bc	0.11	0.68 b
WS-C	0.87 b	1.15	1.94 b	291 b	1.50 c	0.11	0.75 b
WS-SH	1.05 a	1.36	2.31 a	332 a	1.87 b	0.12	1.04 a
WS-OH	0.91 ab	1.24	2.14 ab	307 ab	2.11 a	0.14	0.85 b
2023	0.63 b	1.24	1.87 b	229 b	1.56 b	0.12	1.09 a
2024	1.49 a	1.27	2.71 a	423 a	2.13 a	0.12	0.56 b
T ¹	*	ns	*	*	**	ns	***
Y ¹	***	ns	***	***	***	ns	***
TxY ¹	***	***	***	***	ns	ns	**

Abbreviations: C = control, LA = leaf area, OH = organic hydrogel, SH = synthetic hydrogel, T = treatment, WS = water-stressed, WW = well-watered, Y = year.

¹*, **, and *** indicate significant differences per $p < 0.05$, $**p < 0.01$, and $***p < 0.005$. ns = no difference.

²Different letters within columns indicate significant differences per $p < 0.05$ (SNK test).

TABLE 2 | Dry matter partitioning at the end of 2024 in vines subjected to the soil application of different hydrogels at transplanting, and in control vines.

	Leaves (g DM/vine)	Grapes (g DM/vine)	One-year-old wood (g DM/vine)	Elder wood (g DM/vine)	Roots (g DM/vine)	Aboveground/belowground
WW-C	177	442 a ²	187 ab	135	366 a	2.57 b
WS-C	192	295 c	170 b	130	303 b	2.60 b
WS-SH	226	420 ab	218 a	118	321 ab	3.06 a
WS-OH	210	410 b	205 a	125	367 a	2.59 b ^{**}
P ¹	ns	***	*	ns	**	*

Abbreviations: C = control, DM = dry matter, OH = organic hydrogel, SH = synthetic hydrogel, WS = water-stressed, WW = well-watered.

¹*, **, and *** indicate significant differences per $p < 0.05$, $**p < 0.01$, and $***p < 0.005$. ns = no difference.

²Different letters within columns indicate significant differences per $p < 0.05$ (SNK test).

TABLE 3 | Cane third internode diameter and cane and roots carbohydrate reserves in vines subjected to the soil application of different hydrogels at transplanting, and in control vines, in 2023 and 2024.

	3 rd internode diameter (cm)	3 rd internode soluble sugars (mg/g DM)	3 rd internode starch (mg/g DM)	Roots soluble sugars (mg/g DM)	Roots starch (mg/g DM)
WW-C	1.5 a ²	54.50 a	15.23	46.90 b	60.88
WS-C	1.1 b	48.77 b	16.20	52.42 a	70.05
WS-SH	1.4 a	56.08 a	17.27	52.57 a	65.87
WS-OH	1.3 a	52.36 ab	19.01	50.02 ab	65.74
2023	1.2 b	59.80	7.02	83.76	52.87
2024	1.7 a	37.82	28.24	41.72	63.92
T ¹	**	*	ns	*	ns
Y ¹	***	**	***	***	ns
TxY ¹	ns	***	***	**	***

Abbreviations: C = control, DM = dry matter, OH = organic hydrogel, SH = synthetic hydrogel, T = treatment, WS = water-stressed, WW = well-watered, Y = year.

¹*, **, and *** indicate significant differences per $p < 0.05$, $**p < 0.01$, and $***p < 0.005$. ns = no difference.

²Different letters within columns indicate significant differences per $p < 0.05$ (SNK test).

reduced concentrations of malvidin 3-O-glucoside compared to WW-C and WS-C (Table 6). Additionally, WS-SH showed lower levels of delphinidin 3-O-glucoside and petunidin 3-O-glucoside relative to WW-C and WS-C. Regarding flavonols in grape skins,

no significant differences were observed among treatments for myricetin and kaempferol 3-O-glucoside. However, WS-C and WS-SH exhibited higher quercetin 3-O-glucoside concentrations than WW-C and WS-OH.

just two days (DOY 180–182). In both seasons, the effect of the hydrogels was mild or absent during the initial phase of water stress but became pronounced when the predawn Ψ of WS-C vines fell below -0.8 MPa. Notably, in 2023, WS-OH maintained higher predawn Ψ values than WS-SH, while in 2024, no significant differences were observed between the two treatments. The improved water status under limited irrigation supported the maintenance of higher Leaf A, Leaf E, and WUE (Figure 2), in line with findings from previous studies [18]. Although several authors report improvements in midday stem or leaf Ψ , predawn Ψ is considered the most reliable proxy for soil water potential [43]. Despite the fact that such results need to be confirmed under field conditions, to our knowledge, this is the first *in vivo* study, demonstrating that hydrogel application can influence soil water potential and that observed improvements in plant water status and leaf gas exchanges can be directly attributed to such changes. In our study, we were able to implement a full transition from nonlimiting water supply to severe stress conditions over two consecutive years, although within a relatively short period of 8–12 days, between BBCH 75 and 83. In vineyard conditions, however, water deficit typically develops more gradually and over a longer timeframe than in our pot setup [44]. Within this framework, our data require field validation, but they suggest that effects in the vineyard may be even more pronounced. This is because if the soil Ψ seasonal decline is generally slower than in our experiment, the resulting gap in plant water status could persist for longer periods, potentially amplifying the observed effects, or affecting also earlier or later phenological stages like fruit set, or late ripening. Additionally, the finding that organic hydrogels can produce effects on soil hydrology comparable to those of synthetic hydrogels enhances their appeal and represents a significant step toward the sustainable implementation of hydrogel technology in the field.

The delayed decline in plant water status was the primary factor contributing to the WS-SH and WS-OH postponement or prevention of leaf photoinhibition, compared to WS-C, in both years (Figures 1(c), 1(d)). Proline, a well-known marker of osmotic stress, typically accumulates under severe water deficit, aiding in turgor maintenance and reactive oxygen species (i.e., H_2O_2) scavenging [45]. Taken together, the water status, Fv/Fm, and metabolite data from our study suggest that hydrogel application helps preserve canopy functionality under water stress by delaying turgor loss and limiting reactive oxygen species accumulation in leaves. While more severe or prolonged water deficits might eventually lead to similar effects in WS-SH and WS-OH as those observed in WS-C, our results show that, in both seasons, WS-OH and WS-SH vines recovered full canopy functionality poststress, whereas WS-C vines did not.

By the end of each growing season, WS-SH vines exhibited greater vegetative growth and increased third internode diameter compared to WS-C (Tables 1 and 3). Interestingly, by the end of 2024, both WS-SH and WS-OH treatments also showed higher DM accumulation in one-year-old canes compared to WS-C (Table 2). While WS-C exhibited significantly lower root biomass than WW-C, both WS-SH and WS-OH maintained root DM levels similar to those of WW-C. Overall, hydrogel application supported water-stressed vines' vegetative growth, keeping it closer to that of well-watered vines, consistent with findings in other crops [13, 15, 46]. Notably, only WS-OH produced

a balanced effect on both aboveground and belowground biomass, maintaining a ratio comparable to WW-C. In contrast, WS-SH resulted in a shift toward greater aboveground biomass relative to roots. Although the use of pots may constrain root elongation and these results require confirmation under field conditions and under a higher number of biological replicates, a proportional increase in both canopy and root systems is generally desirable to support long-term water requirements [47]. In this context, WS-OH demonstrated a more favorable response.

The improved vigor and water status observed in WS-SH and WS-OH treatments translated into higher yield per vine compared to WS-C. Specifically, our data show that, by the second year after transplanting, both hydrogels enabled vines subjected to two consecutive seasons of severe water deficit to achieve yields comparable to those of unstressed vines, whereas WS-C vines experienced a 33% reduction in yield, mainly due to a reduction of shoot fruitfulness and bunch weight. A previous study estimated that the cost of applying the same two hydrogels at transplanting would be offset if treated vines produced approximately 3 t/ha more than control vines over the first 3–4 seasons following vineyard establishment [22]. While our findings require confirmation under field conditions and across different water deficit regimes or durations, our results offer a promising outlook. Already in the first productive season, WS-SH vines yielded 3 t/ha more than WS-C, while WS-OH vines exceeded WS-C by 3.3 t/ha. As the vines were dismantled to assess DM partitioning, we were unable to determine whether the observed effects would have persisted in subsequent seasons. However, the higher root mass observed in WS-SH and WS-OH treatments, considering the comparable reserve concentrations, suggests a greater total carbohydrate and nutrient reserves in these vines [48, 49]. This, combined with the increased cane size and higher Leaf N and Leaf K concentrations observed at the end of 2024, supports the hypothesis that the positive effects of hydrogels could extend over multiple growing seasons. Both the replenishment of reserves and vine vigor are known to play crucial roles in determining growth dynamics, inflorescence formation, and overall reproductive performance in the following year [49, 50].

To the best of our knowledge, no studies have assessed the effects of hydrogel application at transplanting on fruit composition in tree crops during the juvenile stages. A few studies have investigated the effects of synthetic hydrogels on mature trees such as citrus, olive, almond, mango, and other woody species [14–16, 23, 51]. In mature grapevines, Ali et al. [20] reported that increasing doses of synthetic hydrogels allowed for a reduction in nitrogen fertilizer application while maintaining adequate yield and satisfactory fruit TSS concentration. However, no studies to date have examined the effects of organic hydrogels on fruit composition in any tree crop, whether applied at transplanting or during the productive stages. In our study, where grapevines were grown in pots and maintained at field capacity for the entire season except for the 12 days of imposed stress, sugar levels remained lower than the typical benchmark for Sangiovese, consistent with findings from many similar studies [31–33]. However, the direct comparison of treatments demonstrates that the application of both synthetic and organic hydrogels at transplanting significantly influences fruit composition. Data suggest that most of these effects are driven by changes in vine vigor

and productivity rather than direct physiological responses to stress. Specifically, the reduced yield observed in WS-C resulted in a marked increase in the leaf-to-fruit ratio (0.776 m²/kg) compared to WW-C (0.619 m²/kg). By maintaining crop load, both WS-SH and WS-OH treatments preserved a lower leaf-to-fruit ratio than WS-C. Therefore, although they maintained a significantly higher rate of photoassimilation during stress than WS-C, these improvements were inherently insufficient to restore sugar and anthocyanin levels to those of either WW-C or WS-C. Indeed, while the increased leaf area per unit of crop in WS-C led to a pronounced increase in grape TSS and TSS/TA ratio [52], comparable to WW-C, WS-SH, and WS-OH lagged behind in grape TSS, total anthocyanins, and phenolics. Considering the different anthocyanins, the overall reduction in their concentration in WS-SH and WS-OH was mainly due to a decrease in malvidin 3-O-G, while other anthocyanidins were not consistently affected, particularly in WS-OH. Therefore, although preliminary and based on potted conditions with a lower TSS baseline than typically observed in the field, our comparative approach demonstrated that the yield increase observed in the first season following hydrogel application inherently altered the leaf-to-fruit ratio of Sangiovese vines. When this ratio is reduced, the higher yield inevitably results in lower ripening levels, even in the presence of better leaf functioning.

Wine composition reflected that of grapes: Wines from WS-SH and WS-OH were notably less alcoholic and less colored than those from WW-C and WS-C, with significantly higher malate and lower phenolic content. Such results confirm that the increased yield at the first productive season comes with a general reduction of alcohol, wine body, structure, and color. At first glance, these effects on fruit and wine composition might seem detrimental for premium red wine production. However, two important considerations should be noted. First, as previously mentioned under our experimental conditions, vines were maintained at field capacity from veraison to harvest, which generally limits TSS and anthocyanin accumulation [53]. In field conditions, prolonged water stress typically accelerates ripening, especially in young vines with limited root development, leading to excessive sugar accumulation [54, 55]. Second, current trends in the wine industry favor wines with lower alcohol levels [56, 57]. In this context, although appropriate field experiments are needed to validate these findings under operational conditions, our data suggest that hydrogel application at transplanting can delay ripening and slow sugar accumulation in young vines, an effect that is eventually desirable for the production of light-bodied red wines, and optimal in the case of white and sparkling wines [58, 36]. Within this framework, our work suggests that ripening should be carefully monitored in hydrogel-treated vines. Growers may also consider temporarily adjusting the wine style during the first season, compared with situations where hydrogels are not applied, favoring lighter and fresher profiles until the varietal's typical leaf-to-fruit ratio is re-established. Further studies will be essential to confirm these effects under field conditions.

5 | Conclusions

Hydrogels are gaining interest among growers of various crops as a response to climate change and increasing temperatures. In vineyards, their incorporation at transplanting appears promising for mitigating summer stress, reducing vine mortality, and accelerating the transition to productive stages. In potted vines, both synthetic and organic hydrogels improved

vine water status under progressive water deficit, enhanced growth over two seasons compared to stressed controls, and, by the end of the second season, maintained yields comparable to unstressed vines. In contrast, stressed controls showed reduced crop size and increased leaf-to-fruit ratios, which resulted in accelerated ripening and higher wine alcohol content, compared to hydrogel-treated stressed vines, which lagged behind all the other treatments in terms of grape sugars, wine alcohol, anthocyanins, and phenolics. Notably, no significant differences were observed between vines treated with organic versus synthetic hydrogels, highlighting the potential of organic-derived hydrogels to provide similar benefits. Although further studies under field conditions and alternative experimental designs are necessary to confirm and expand upon these findings, our study lays the groundwork for the sustainable implementation of organic hydrogels in vineyard management.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The authors will make data available upon request.

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Supporting Information

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

Supporting 1. Supporting Figure 1—Effects of treatments and years on main shoot leaf area (panel a), lateral shoot leaf area (panel b), total vine leaf area (panel c), and pruning weight (panel d) of vines subjected to the soil application of different hydrogels at transplanting and in control vines. Means \pm standard errors ($n = 4$). Different letters indicate significant differences between treatments per $p < 0.05$ (SNK test). WW = well-watered; WS = water-stressed; C=control; SH = synthetic hydrogel; OH = organic hydrogel.

Supporting 2. Supporting Figure 2—Effects of treatments and years on cane third internode (panels a and b) and roots (panels c and d) soluble sugars (panels a and c) and starch (panels b and d) in vines subjected to the soil application of different hydrogels at transplanting, and in control vines. Means \pm standard errors ($n = 4$). Different letters indicate significant differences between treatments per $p < 0.05$ (SNK test). WW = well-watered; WS = water-stressed; C=control; SH=synthetic hydrogel; OH= organic hydrogel.