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Research paper

Grapevine responses to water deficit and N x K fertilization: Seasonal variation of 'Cabernet Sauvignon' and 'Grenache' physiology

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ABSTRACT

Water and nutrient availability strongly influence grapevine yield and wine quality. While the single effects of water deficit and nutrient deficiency have been extensively studied, their interaction remains poorly understood. Two-year-old *Vitis vinifera* L. cv 'Cabernet Sauvignon' (near-anisohydric) and 'Grenache' (near-isohydric) plants grafted on SO4 were grown in pots under semi-environmental conditions. Plants were either maintained well-watered (100 % lysimeter evapotranspiration, ET_{lys}) or subjected to water deficit (33 % ET_{lys}) and treated with different nitrogen (N) and potassium (K) fertilization rates. Morpho-physiological analyses revealed that water deficit significantly reduced plant growth, with 'Grenache' exhibiting a slower growth rate than 'Cabernet Sauvignon'. At flowering, water treatment was the dominant factor modulating plant response. Water deficit reduced stomatal conductance (g_s), while enhancing accumulation of several nutrients. N and K fertilization became dominant factors during the following developmental stages: leaf ionomic composition of 'Cabernet Sauvignon' was responsive to N and K from veraison, and this behavior aligns with g_s reduction caused by high N rather than water deficit. Conversely, the ionomic composition of 'Grenache' was affected by N and K only at maturity, while g_s was consistently modulated by water availability throughout the season. K effects on leaf ionomic composition were more pronounced under high N. We highlighted the importance of both water and chemical inputs, whose effects vary with cultivar and developmental stage. Results will contribute to the improvement of viticultural sustainability by developing optimized fertilization strategies tailored to plant requirements under specific environmental conditions.

1. Introduction

Among abiotic stressors, water scarcity is a major limiting factor for viticultural production in arid and semi-arid regions, such as the Mediterranean basin, where prolonged summer droughts often occur (Dayer et al., 2019; Naulleau et al., 2021). The rise of atmospheric greenhouse gas emissions and the associated climate change are predicted to increase both the frequency and intensity of drought events (IPCC, 2023). In this context, improving water use efficiency has become a main goal for modern agriculture (Escalona et al., 2013).

Grapevine (*Vitis vinifera* L.), a moderately drought-tolerant species, is commonly cultivated in Mediterranean-like climates such as France, Spain, and Italy. In these regions, vines are traditionally grown without supplemental irrigation (Lovisolo et al., 2010), which is often applied to maximize yield and profitability (Sivilotti et al., 2024). Regulated Deficit Irrigation (RDI) has emerged as a promising strategy to control vegetative growth and optimize plant water use, helping enhance both viticultural sustainability and berry quality (Dayer et al., 2019; Sivilotti et al., 2024). However, excessive water limitation can impair photosynthesis and transpiration, leading to reduced plant growth (Lavoie-Lamoureux et al., 2017; Seleiman et al., 2021). Under severe drought conditions, physiological dysfunctions, such as xylem

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Abbreviations: A, net photosynthesis; C, carbon; Ca, calcium; Cu, copper; CS, Cabernet Sauvignon; E, transpiration rate; ET_{lys}, lysimeter evapotranspiration; Fe, iron; g_s,, stomatal conductance; GR, Grenache; iWUE, intrinsic water use efficiency; K, potassium; Mg, magnesium; Mn, manganese; N, nitrogen; Na, sodium; P, phosphorous; RDI, regulated deficit irrigation; S, sulfur; WD, water deficit; WW, well-watered; Zn, zinc; Ψ_{stem}, stem water potential.

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cavitation, may occur (Lamarque et al., 2023; Zwieniecki and Secchi, 2015).

Water stress sensitivity varies among *Vitis vinifera* cultivars (Bota et al., 2016; Pou et al., 2012; Schultz, 2003). Indeed, grapevines are often classified as isohydric or anisohydric cultivars, depending on their stomatal sensitivity to water shortage. Isohydric cultivars exhibit early stomatal closure in response to small decreases in soil water content, modifying their growth by limiting carbon assimilation, whereas anisohydric ones tend to maintain their stomata open, reaching more negative water potential values under increasing soil dehydration (Herrera et al., 2022; Tardieu and Simonneau, 1998). However, cultivars do not have a fixed behavior and can show plasticity in their drought response strategies depending on environmental conditions, stress intensity, and duration (Hochberg et al., 2018; Morabito et al., 2022). Moreover, it remains unclear which stomatal behavior confers greater adaptive advantages under future climate change scenarios (Charrier et al., 2018; Gambetta et al., 2020; Linhart et al., 2023; Nerva et al., 2023). Therefore, irrigation management must consider multiple variables, including genotype, phenological stage, and local environmental and edaphic conditions.

In addition to water, nitrogen (N) and potassium (K) are key macronutrients that influence vine development, physiological efficiency, and grape quality (Keller, 2020; Verdenal et al., 2021; Villette et al., 2020). N is a fundamental component of amino acids, proteins, and nucleic acids, while K plays crucial roles in enzyme activation, pH homeostasis, and cellular osmoregulation (Clarkson and Hanson, 1980; Marschner, 2011), suggesting its involvement in drought resilience (Cakmak, 2005; Shabala and Pottosin, 2014; Sperling et al., 2024). Although N fertilization can promote shoot growth and photosynthetic efficiency, excessive applications may increase plant water demand, potentially exacerbating drought sensitivity (Metay et al., 2014; Plavcová et al., 2013; Verdenal et al., 2021). Moreover, N fertilization can influence rhizospheric pH, hence affecting the bioavailability of other nutrients, especially micronutrients (McClure et al., 1990). In turn, excessive K accumulation in berries may result in a decrease in wine acidity, compromising the overall wine quality (Kodur, 2011; Villette et al., 2020). Consequently, fertilization practices must be carefully adjusted according to the vine nutritional and water needs to minimize environmental losses (Faralli et al., 2023).

Transpiration-driven mass flow is a primary mechanism for nutrient movement in soil (Cramer et al., 2009; Faralli et al., 2023), particularly for nitrate (NO_3^-). Hence, the reduction in soil moisture can limit nutrient mobility and uptake from roots (Gonzalez-Dugo et al., 2010; Keller, 2020; Marschner, 2011). Previous studies showed contrasting results regarding K^+ concentrations under water deficit, with some reporting higher levels under well-watered conditions (Rogiers et al., 2017) while others indicating increases under drought stress (Rivas-Ubach et al., 2012). Moreover, limited water availability reduces plant growth, potentially influencing N demand (Gonzalez-Dugo et al., 2005). Despite the increasing interest in the individual roles of water and nutrient management, their combined effects on vine water and nutritional status have not been extensively explored (Brueck, 2008; Taskos et al., 2020).

This study aimed to investigate the physiological and ionomic responses of two grapevine cultivars, ‘Cabernet Sauvignon’ and ‘Grenache’, to different irrigation and N x K fertilization regimes throughout the growing season. The two cultivars were chosen based on the reported contrasting hydraulic behaviors, as ‘Cabernet Sauvignon’ is often classified as anisohydric and ‘Grenache’ as isohydric (Keller, 2020; Lovisolo et al., 2010), making them ideal for assessing varietal responses to environmental conditions. Moreover, ‘Cabernet Sauvignon’ is one of the most cultivated vine varieties, while ‘Grenache’ (also known as Garnacha or Cannonau) is extremely drought resistant and mainly cultivated in Spain and France (OIV, 2017). By assessing the seasonal dynamics of vine water status, gas exchange parameters, and nutrient composition, the complex genotype-dependent interactions between water deficit and nutrient management have been investigated. Our findings provide valuable insights for optimizing irrigation and fertilization practices under changing climatic conditions, with direct implications for sustainable vineyard management.

2. Materials and methods

2.1. Experimental site and design

The experiment was conducted in the experimental field of the University of Udine “A. Servadei” (Udine, Italy) (46020 N, 13130 E; 88 m a.s.l.). Supplementary Figure S1 shows the daily precipitation (mm) and mean temperatures (°C) collected from March to September by a weather station located in the experimental farm (ARPA FVG – OSMER, http://osmer.fvg.it). On March 24, 2023, 192 two-year-old vines (*Vitis vinifera* L.) cv. ‘Cabernet Sauvignon’ and ‘Grenache’, both grafted onto SO4 rootstock (and previously grown for one year in field) were potted (20 L) in a mixture of soil collected at the farm site with a sandy-loam texture (2 cm-sieved, 12 %, 40 %, and 48 % of clay, silt, and sand, respectively) supplemented with peat (10 %, Brill®1, Agrochimica, Bolzano, Italy). Supplementary Table S1 shows results from soil analysis carried out by the external laboratory of ERSA before the experiment.

The experiment was performed in semi-environmental conditions under a tunnel with open sides covered with polyethylene film, as described in Vuerich et al. (2021). Vines were arranged in five single rows with a NW-SE orientation. The vines were pruned to five buds, and the emerging shoots were then trained using the vertical shoot positioning system. The number of bunches per pot was restricted to two after budburst. Budburst started on average on April 12, 2023.

A $2 \times 2 \times 2 \times 2$ factorial (2 water regimes, 2 N treatments, 2 K treatments, 2 cultivars) completely randomized block design with 4 replicates was used (Supplementary Figures S2 and S3). In detail, the factors were: a) vine cultivars (=2): ‘Cabernet Sauvignon’ (CS) and ‘Grenache’ (GR), b) water regimes (=2): well-watered (WW) and water deficit (WD), c) nitrogen (N) treatments (=2): high-N fertilization (100 % N, +N) and low-N fertilization (30 % N), d) potassium (K) treatments (=2): high-K fertilization (100 % K, +K) and low-K fertilization (30 % K), e) 4 plots made by three vines within each treatment were considered for sampling, for a total of 12 grapevine pots per condition. Two additional buffer plants were placed at each row head to avoid border effects.

2.2. Water deficit and N and K treatment

Before water treatment imposition, all vines were fully irrigated to maintain field capacity. Water was supplied via a drip irrigation system with two emitters per pot ($2\,L \bullet h^{-1}$ PCJ, Netafim, Harzerim, Israel), and irrigation was supplied every day at 5:00 am. The water regime started to be differentiated (WW and WD) on May 16, 2023 (T3) and continued until harvest (September 6, 2023, T4). Four WW and four WD pots were placed on self-constructed scales functioning as lysimeters, as previously described by Hochberg et al. (2017a). Briefly, the daily *lysimeter evapotranspiration* (ET_{lys}) was calculated as the mean daily mass loss of WW potted vines. WW plants were irrigated with 100 % of ET_{lys}, while WD plants received 33 % of ET_{lys}, by placing three and one emitters on WW and WD pots, respectively.

At complete budburst (April 17, 2023, T1), all vines were provided with mineral nutrition (2.5 g of N-P-K Nitrophoska® and 6.375 g of superphosphate 20 P). After a week (T2), different doses of calcium nitrate ($Ca(NO_3)_2$ 15.5 N) and potassium sulfate (K_2SO_4 50 K) were applied (Supplementary Table S2). The same fertilization was repeated at flowering (June 5, 2023, T4). $Ca(NO_3)_2$ was supplemented over two weeks to prevent nitrate leaching.

2.3. Morpho-physiological measurements and plant water status

Every two weeks and throughout the experiment the following measurements were conducted: shoot growth rate, SPAD index, midday stem water potential, and leaf gas exchange analyses.

Shoot growth rate was calculated by the sum of the lengths of the two main shoots per plant using a measuring tape ($N = 12$). Plant N status was evaluated using a portable chlorophyll meter (SPAD-502, Konica-Minolta, Osaka, Japan). Measurements were carried out between the fifth and the seventh fully expanded leaves starting from the apex ($N = 3$).

On sunny days between 11:30 am and 02:00 pm, plant water status was assessed by *midday stem water potential* (Ψ_{stem}) and gas exchange parameters. Measurements were performed on three fully expanded, well-exposed leaves (at node positions 6–8 from the base) per replicate plant ($N = 3$ plants per treatment). To avoid potential effects of repeated sampling on leaf physiology, different leaves were selected at each measurement date. The three replicates were randomly chosen out of the 12 plants per treatment, ensuring that each plant was measured at least once during the season. To measure Ψ_{stem}, leaves were bagged and covered with aluminum foil at least one hour before measurement. Afterward, leaves were placed in a Scholander-type pressure chamber (Soil Moisture Co., Santa Barbara, USA) with the petiole excised using a razor blade and pressurized using a nitrogen tank. Ψ_{stem} was determined when the xylem sap emerged from the cut end of the petiole.

Leaf gas exchange parameters, namely *stomatal conductance* (g_s, mol H_2O $m^{-2} \bullet s^{-1}$), *transpiration rate* (E, mmol H_2O $m^{-2} \bullet s^{-1}$), and *net photosynthesis* (A, µmol CO_2 $m^{-2} \bullet s^{-1}$) were measured using an infrared gas analyzer (LI-6400XT, LiCor, Inc., Lincoln, NE, USA). Measurements were taken at a constant light intensity (1000 µmol $m^{-2} \bullet s^{-1}$), CO_2

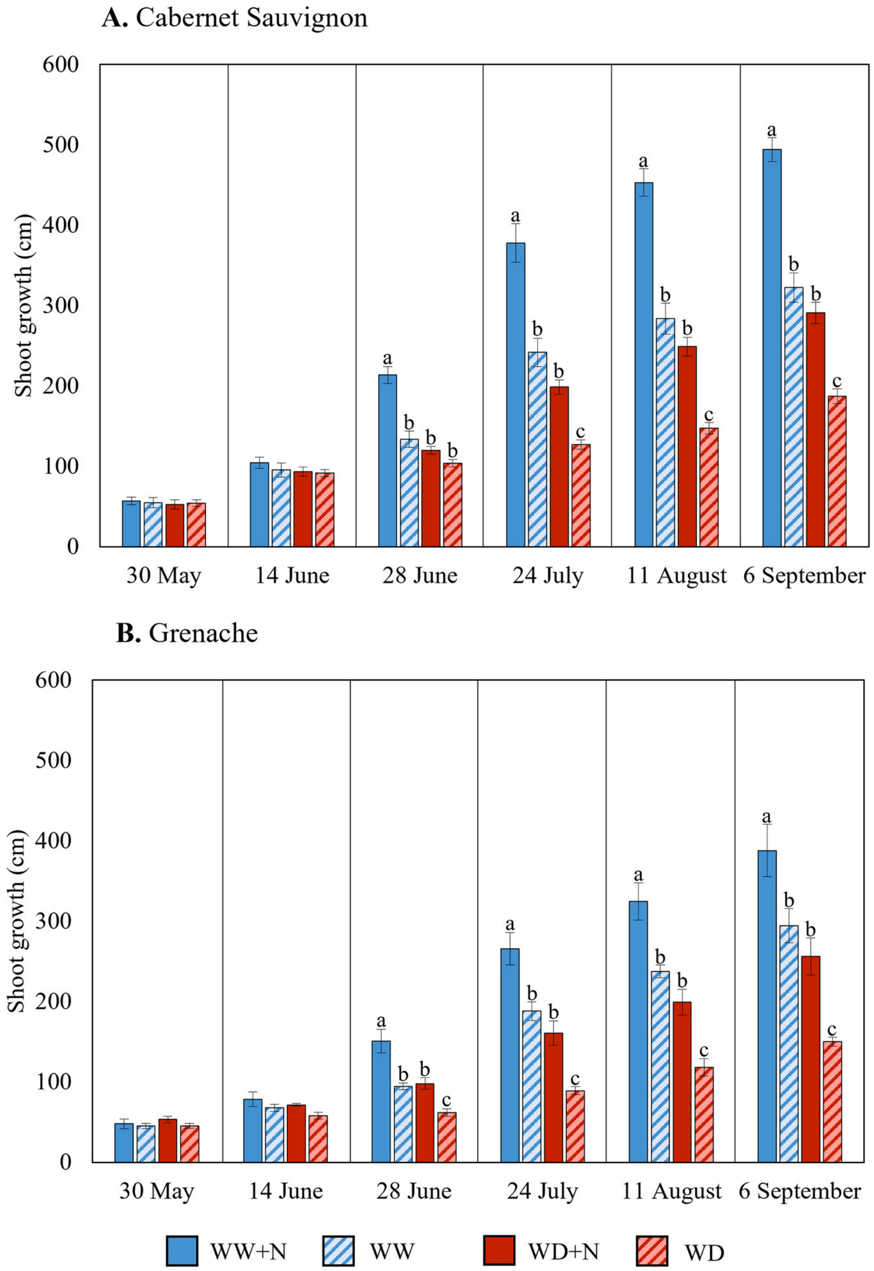

Fig. 1. Shoot length (cm) of 'Cabernet Sauvignon' (**A**) and 'Grenache' (**B**) measured along the season. Results are shown as mean values ± SE. Letters represent significant differences between treatments of same cultivar and same date (one-way ANOVA and, when the test was significant, means were separated with Tukey HSD test, $N = 12$, $p < 0.05$). WW+N: plants under 100 % ET_{lys} and 100 % N; WW: plants under 100 % ET_{lys} and 30 % N; WD+N: plants under 33 % ET_{lys} and 100 % N; WD: plants under 33 % ET_{lys} and 30 % N.

concentration (400 μmol • mol^{-1}), and ambient temperature and humidity. The *intrinsic water use efficiency* (iWUE) was calculated by dividing A by g_s.

2.4. Leaf elemental analyses

At flowering (June 9, 2023, S1), veraison (August 7, 2023, S2), and maturity (September 7, 2023, S3) stages, three fully-expanded (at node positions 6–8 from the base) leaves *per* treatment were collected to evaluate elemental concentrations, in particular calcium (Ca), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), phosphorous (P), sulfur (S), and zinc (Zn). Nutrients were determined by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES 5800, Agilent Technologies, Santa Clara, USA). In addition, total N and C contents were determined by CHN analyzer (CHN-IRMS Isoprime 100 Stable Isotope Ratio Mass Spectrometer, Elementar, Como, Italy). For ICP-OES analyses, a pool of three leaves cut in half were oven-dried for 2 weeks at 60 °C and grounded. For each sample, 100 mg of ground powder was ashed at 550 °C in glass vials and digested in ultrapure HNO_3. Element quantification was performed using certified multi-element standards.

2.5. Statistical analyses

The effects of measurement date, cultivar, water treatment, N treatment, K treatment, and their interactions were evaluated by means of a five-way ANalysis OF VAriance (ANOVA, Supplementary Tables S3 and S6). Since K treatment did not significantly affect morphological and physiological parameters, this factor is not shown in the figures of shoot growth, Ψ_{stem}, g_s, and iWUE (Figs. 1–3); figures including all treatments are provided in Supplementary Material (Supplementary Figures S5-S9). To assess the effects of measurement date, water treatment, N treatment, and their interaction on the morpho-physiological measurements of each cultivar, a three-way ANOVA was also performed (Supplementary Tables S4 and S5). Additionally, a one-way ANOVA was conducted to evaluate the effects of the treatments within each date of measurement and cultivar. For significant tests (p-value < 0.05), Tukey's HSD post hoc test was performed. The analyses of variance and graphs were performed using R statistical software (R Core Team, 2023, version 4.2.2). To graphically summarize the specific responses of the two cultivars at each developmental stage regarding the elemental content, heatmaps and Principal Component Analyses (PCAs) were generated using SRPlot (https://www.bioinformatics.com.cn/en, Tang et al., 2023) webtool with default parameters.

3. Results

3.1. Vegetative development

From late June, shoot length was significantly affected by water deficit, regardless of N treatment and cultivar (Fig. 1). By the end of the season, 'Cabernet Sauvignon' plants under water deficit exhibited an approximately 40 % reduction in shoot length compared to well-watered plants. This effect was particularly evident in 'Grenache', in which the shoot length of WD plants was reduced by almost 50 % compared to WW plants.

Conversely, the high N supply increased 'Cabernet Sauvignon' and 'Grenache' shoot growth, independent of the water treatment. In 'Cabernet Sauvignon', N increased plant growth by approximately 50 % under well-watered conditions, with WW+N plants reaching the highest values recorded.

'Grenache' exhibited a significantly lower growth rate compared to 'Cabernet Sauvignon', as shown by the significant effect of cultivar in the five-way ANOVA results ($p < 0.001$, Supplementary Table S3). In September, WW+N 'Grenache' plants reached an average shoot length of 388 cm, compared to 494 cm measured in WW+N 'Cabernet Sauvignon' plants.

3.2. Vine water status

Water deficit reduced stem water potential (Ψ_{stem}) compared with vines grown under well-watered conditions (Fig. 2). Nevertheless, Ψ_{stem} never dropped to critical values, with 'Cabernet Sauvignon' and 'Grenache' maintaining Ψ_{stem} always above −0.67 and −0.71 MPa,

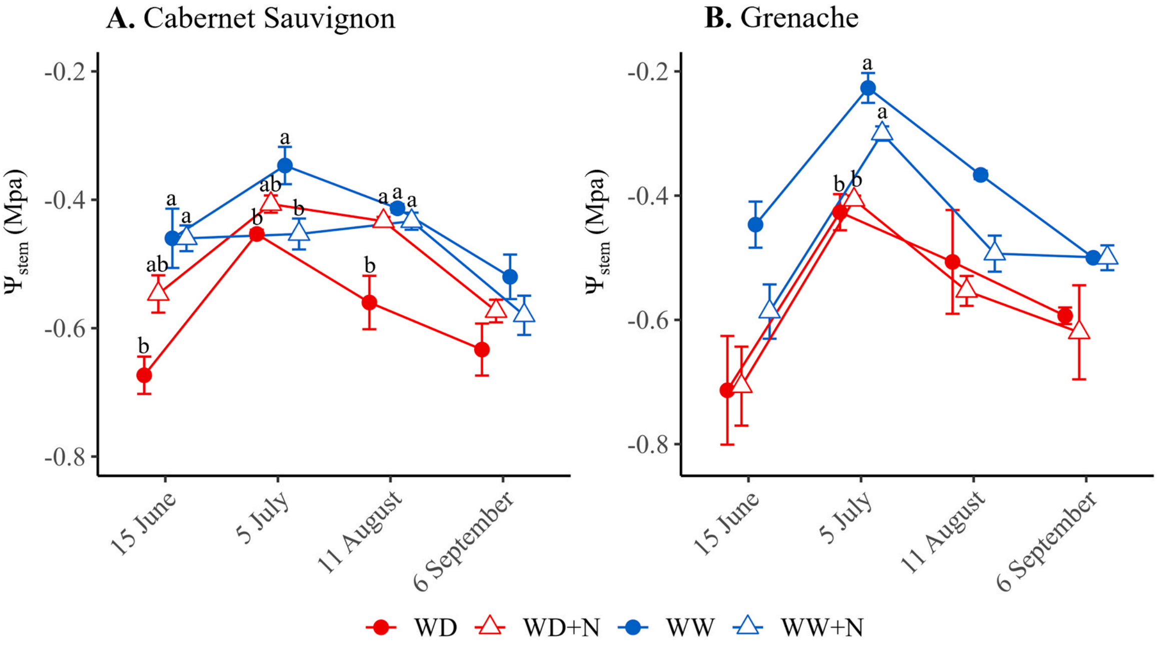

Fig. 2. Midday stem water potential (Ψ_{stem}, MPa) of 'Cabernet Sauvignon' (**A**) and 'Grenache' (**B**) measured along the season. Results are shown as mean values ± SE. Letters represent significant differences between treatments of same cultivar and same date (one-way ANOVA and, when the test was significant, means were separated with Tukey HSD test, N = 3, $p < 0.05$). WW+N: plants under 100 % ET_{lys} and 100 % N; WW: plants under 100 % ET_{lys} and 30 % N; WD+N: plants under 33 % ET_{lys} and 100 % N; WD: plants under 33 % ET_{lys} and 30 % N.

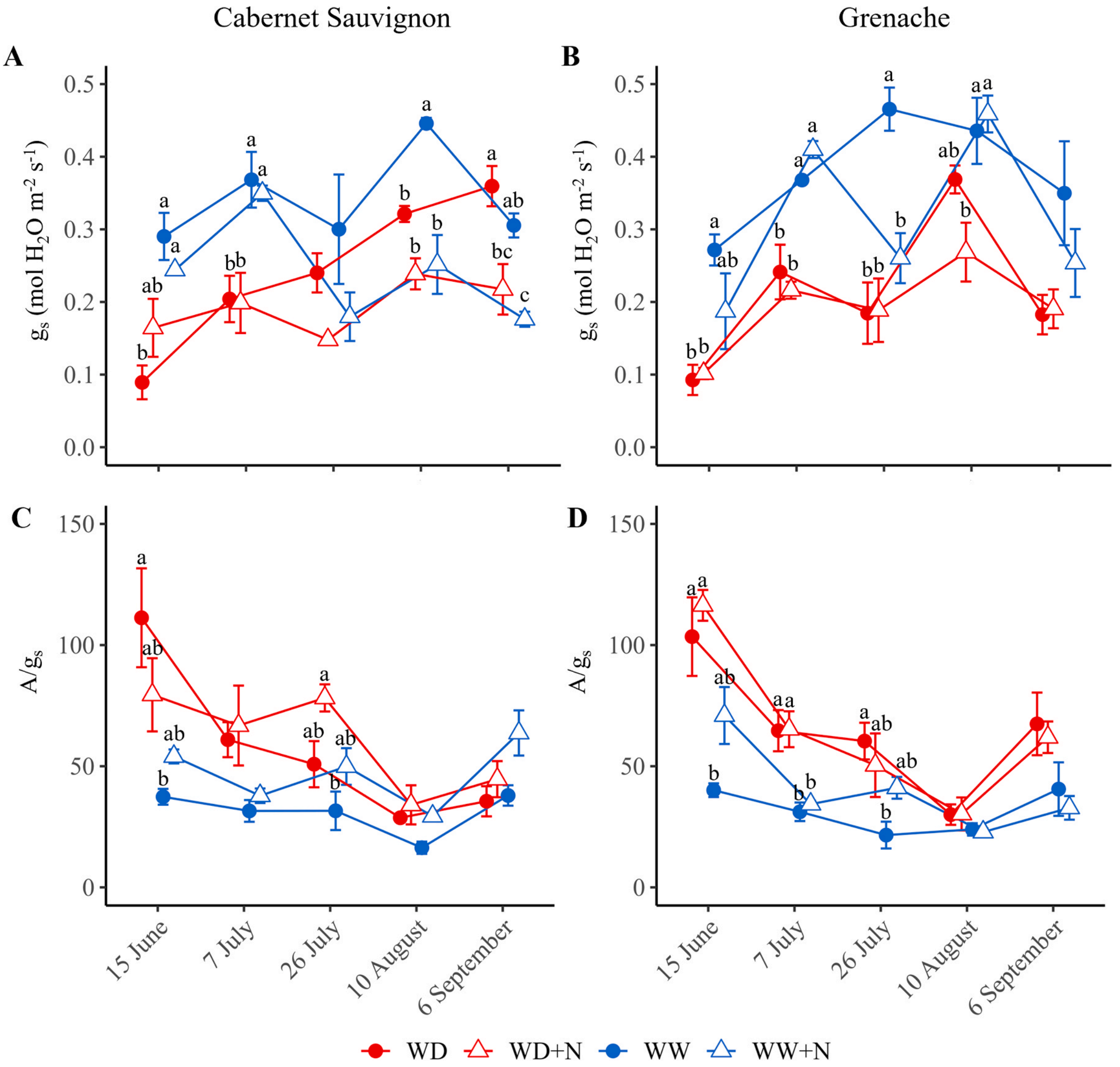

Fig. 3. Stomatal conductance (g_s, mol H_2O m^{-2} • s^{-1}) and water-use efficiency (iWUE, µmol CO_2 • mol^{-1} H_2O) of 'Cabernet Sauvignon' (**A** and **C**) and 'Grenache' (**B** and **D**) measured along the season. Results are shown as mean values ± SE. Letters represent significant differences between treatments of same cultivar and same date (one-way ANOVA and, when the test was significant, means were separated with Tukey HSD test, N = 3, $p < 0.05$). WW+N: plants under 100 % ET_{lys} and 100 % N; WW: plants under 100 % ET_{lys} and 30 % N; WD+N: plants under 33 % ET_{lys} and 100 % N; WD: plants under 33 % ET_{lys} and 30 % N.

respectively. In 'Grenache', significant differences in Ψ_{stem} among water treatments were observed in July. The minimum Ψ_{stem} was recorded during early-season measurements (June 15), while maximum values were recorded in July, after which Ψ_{stem} decreased progressively throughout the season.

Notably, a positive effect of N treatment under water deficit conditions was observed in 'Cabernet Sauvignon', where WD+N plants exhibited Ψ_{stem} more similar to those recorded in well-watered plants than in WD plants. On the contrary, under well-watered conditions, both cultivars showed the highest Ψ_{stem} under low N supply, while WW+N reached values comparable to those of water deficit plants in July and September for 'Cabernet Sauvignon', and in June and August for 'Grenache'.

3.3. Leaf gas exchange parameters

Results from the five-way ANOVA revealed that the cultivar had a significant effect on net assimilation rate (A) ($p = 0.016$), stomatal conductance (g_s) ($p = 0.007$), and transpiration rate (E) ($p = 0.011$) (Supplementary Table S3). As observed for Ψ_{stem}, the highest g_s values were found in WW plants, reaching maximum values in August (Figs. 3**A**, 3**B**).

Overall, the physiological response of 'Grenache' was primarily modulated by water treatment. Water deficit reduced g_s, with the lowest values recorded during the earliest water deficit imposition (June 15): WD plants exhibited g_s values that were one-third of those in WW plants. The intrinsic water use efficiency (iWUE) was higher under water deficit conditions (Figs. 3**C**, 3**D**). This effect was especially evident at the beginning of the season, when iWUE in WD plants was almost three times higher than that of WW plants. During the last experimental period, similar iWUE values were observed among different treatments. Conversely, plants subjected to different N supplies showed similar g_s and iWUE, except for WW+N plants, which showed noticeably lower g_s on July 26.

'Cabernet Sauvignon' displayed a different trend compared to 'Grenache'. Early season measurements of gas exchange indicated that water deficit caused a significant reduction in g_s. On June 15, iWUE in WD plants was three times higher than that of WW plants. On the contrary, from July 26 onward, g_s was negatively affected by N treatment rather than water treatment, thereby increasing iWUE. This effect was especially evident during the latest season measurements (September), when both WW and WD plants displayed significantly higher g_s than

WW+N and WD+N plants. As an example, on August 10, WW+N plants displayed a g_s of 0.25, compared to 0.44 in WW plants. These findings are consistent with the results of the three-way ANOVA, in which N treatment had a stronger impact on 'Cabernet Sauvignon' than on 'Grenache'. Moreover, unlike 'Grenache', a significant interaction between measurement date and water treatment, and between measurement date and N treatment, was observed only in 'Cabernet Sauvignon' (Supplementary Tables S4 and S5).

3.4. *Elemental composition*

Results from the five-way ANOVA show that sampling dates and cultivars had a significant effect on all elemental concentrations except for Fe (with respect to the sampling date) and Cu and P (with respect to the cultivar) (Supplementary Table S6). Consequently, leaf elemental concentrations were analyzed separately for each sampling date and cultivar (Supplementary Tables S7, S8, and S9). At flowering, water treatment had the most pronounced effect, significantly influencing several macro- (K, Mg, P, and S) and micronutrients (Cu, Fe, Mn, and

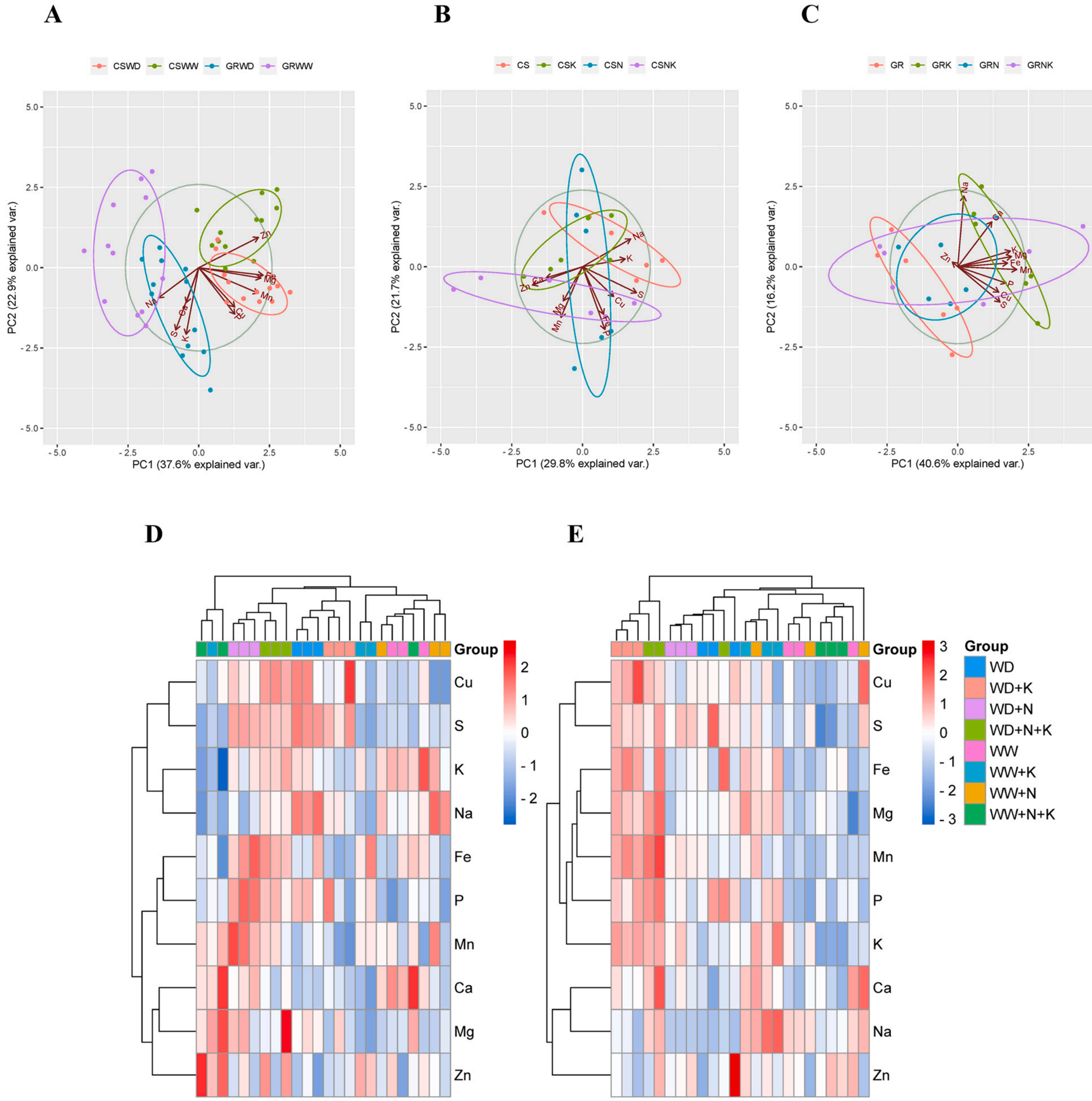

Fig. 4. Elemental concentration of grapevine leaves collected at flowering (S1). Principal component analyses (PCAs) of Cabernet Sauvignon and Grenache cultivars grouped by water treatment (**A**; CSWD or CSWW: Cabernet Sauvignon under 33 % ET_{lys} or 100 % ETlys, respectively; GRWD or GRWW: Grenache under 33 % ET_{lys} or 100 % ET_{lys}, respectively; irrespective of N and K treatments), and Cabernet Sauvignon (**B**; CS: Cabernet Sauvignon under 30 % N + 30 % K; CSK: Cabernet Sauvignon under 30 % N + 100 % K; CSN: 'Cabernet Sauvignon' under 100 % N + 30 % K; CSNK: Cabernet Sauvignon under 100 % N + 100 % K, irrespective of water treatment) and 'Grenache' (**C**; GR: Grenache under 30 % N + 30 % K; GRK: Grenache under 30 % N + 100 % K; GRN: Grenache under 100 % N + 30 % K; GRNK: Grenache under 100 % N + 100 % K, irrespective of water treatment) samples grouped by fertilization treatment. Heatmaps show hierarchical clustering of elemental concentration of Cabernet Sauvignon (**D**) and Grenache (**E**) samples (N = 3). WD, water deficit 30 % N + 30 % K; WW, well-watered 30 % N + 30 % K; WD+K, water deficit 30 % N + 100 % K; WW+K, well-watered 30 % N + 100 % K; WD+N, water deficit 100 % N + 30 % K; WW+N, well-watered 100 % N + 30 % K; WD+N+K, water deficit 100 % N + 100 % K; WW+N+K, well-watered 100 % N + 100 % K.

Na). Contrarily, N and K treatments significantly affected the concentration of fewer elements, with N treatment influencing Ca, N, and Mn concentrations, and K treatment affecting Mg, P, S, Fe, and Zn concentrations (Supplementary Table S10).

In contrast, at maturity, all elemental concentrations except C and Cu were significantly influenced by N treatment, whereas water and K treatments modulated fewer elements, with K treatment primarily altering the concentration of macronutrients (C, Ca, K, Mg, and S) and water treatment mainly influencing micronutrients (Fe, Mn, Na, and Zn) (Supplementary Table S11).

These trends were further confirmed by principal component analyses (PCAs) and clustering heatmaps. At flowering, samples separated primarily according to cultivar along PC1 (37.6 %) and to water treatment along PC2 (22.9 %, Fig. 4A). No clear sample separation was observed according to N and K treatments at this stage, except for GR that separated from GR+K along PC1 (40.6 %, Fig. 4C). Water deficit led to an increase in the concentration of several nutrients compared to well-watered conditions. This effect is particularly evident in 'Grenache'

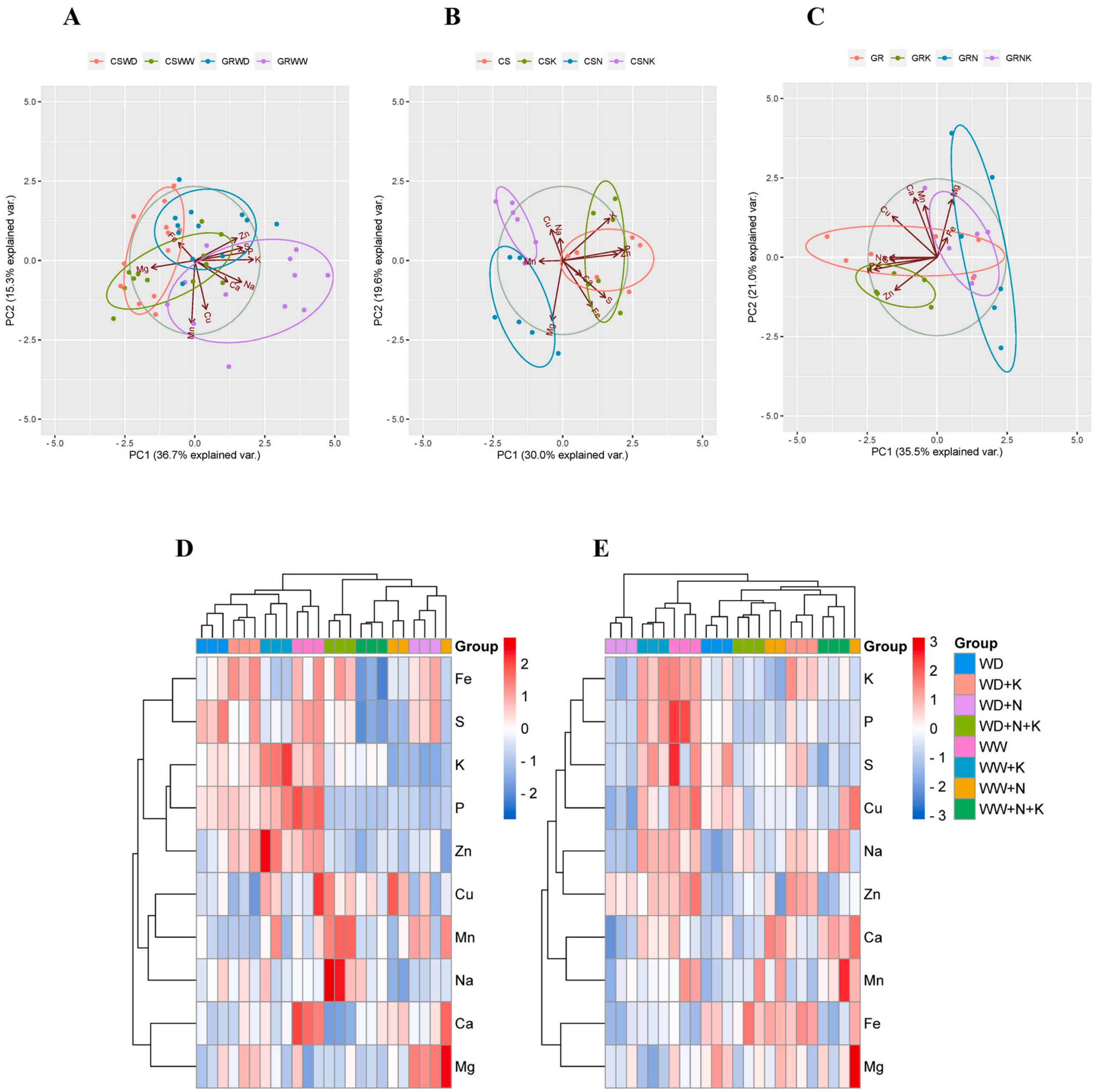

Fig. 5. Elemental concentration of grapevine leaves collected at veraison (S2). Principal component analyses (PCAs) of Cabernet Sauvignon and Grenache cultivars grouped by water treatment (**A;** CSWD or CSWW: Cabernet Sauvignon under 33 % ET_{lys} or 100 % ET_{lys}, respectively; GRWD or GRWW: Grenache under 33 % ET_{lys} or 100 % ET_{lys}, respectively; irrespective of N and K treatments), and Cabernet Sauvignon (**B**; CS: Cabernet Sauvignon under 30 % N + 30 % K; CSK: Cabernet Sauvignon under 30 % N + 100 % K; CSN: 'Cabernet Sauvignon' under 100 % N + 30 % K; CSNK: Cabernet Sauvignon under 100 % N + 100 % K, irrespective of water treatment) and 'Grenache' (**C**; GR: Grenache under 30 % N + 30 % K; GRK: Grenache under 30 % N + 100 % K; GRN: Grenache under 100 % N + 30 % K; GRNK: Grenache under 100 % N + 100 % K, irrespective of water treatment) samples grouped by fertilization treatment. Heatmaps show hierarchical clustering of elemental concentration of Cabernet Sauvignon (**D**) and Grenache (**E**) samples (N = 3). WD, water deficit 30 % N + 30 % K; WW, well-watered 30 % N + 30 % K; WD+K, water deficit 30 % N + 100 % K; WW+K, well-watered 30 % N + 100 % K; WD+N, water deficit 100 % N + 30 % K; WW+N, well-watered 100 % N + 30 % K; WD+N+K, water deficit 100 % N + 100 % K; WW+N+K, well-watered 100 % N + 100 % K.

under high N and K treatments, where WD + N + K plants exhibited significantly higher levels of K, Mn, P, and S than WW + N + K plants (Supplementary Table S7).

Conversely, PCAs and heatmaps at veraison (especially for 'Cabernet Sauvignon', Fig. 5) and maturity (both 'Cabernet Sauvignon' and 'Grenache', Fig. 6) show that plants separated primarily according to N treatment, followed by K treatment. At veraison, 'Cabernet Sauvignon' samples separated firstly according to N treatment along PC1 (30.0 %), and secondly according to K treatment along PC2 (19.6 %), especially under high N supply (Fig. 5**B**). 'Grenache' clustering was less evident, although separation between 100 % N + 100 % K and 30 % N + 100 % K was still visible along PC1 (35.5 %, Fig. 5**C**). At maturity, 'Cabernet Sauvignon' samples remained separated mainly according to N treatment along PC1 (38.4 %, Fig. 6**B**). In 'Grenache', PC1 (41.2 %) captured sample separation according to N treatment, while PC2 (23.6 %) captured separation according to K treatment, although only under high N (Fig. 6**C**).

Mineral concentrations tended to be lower under high N compared to

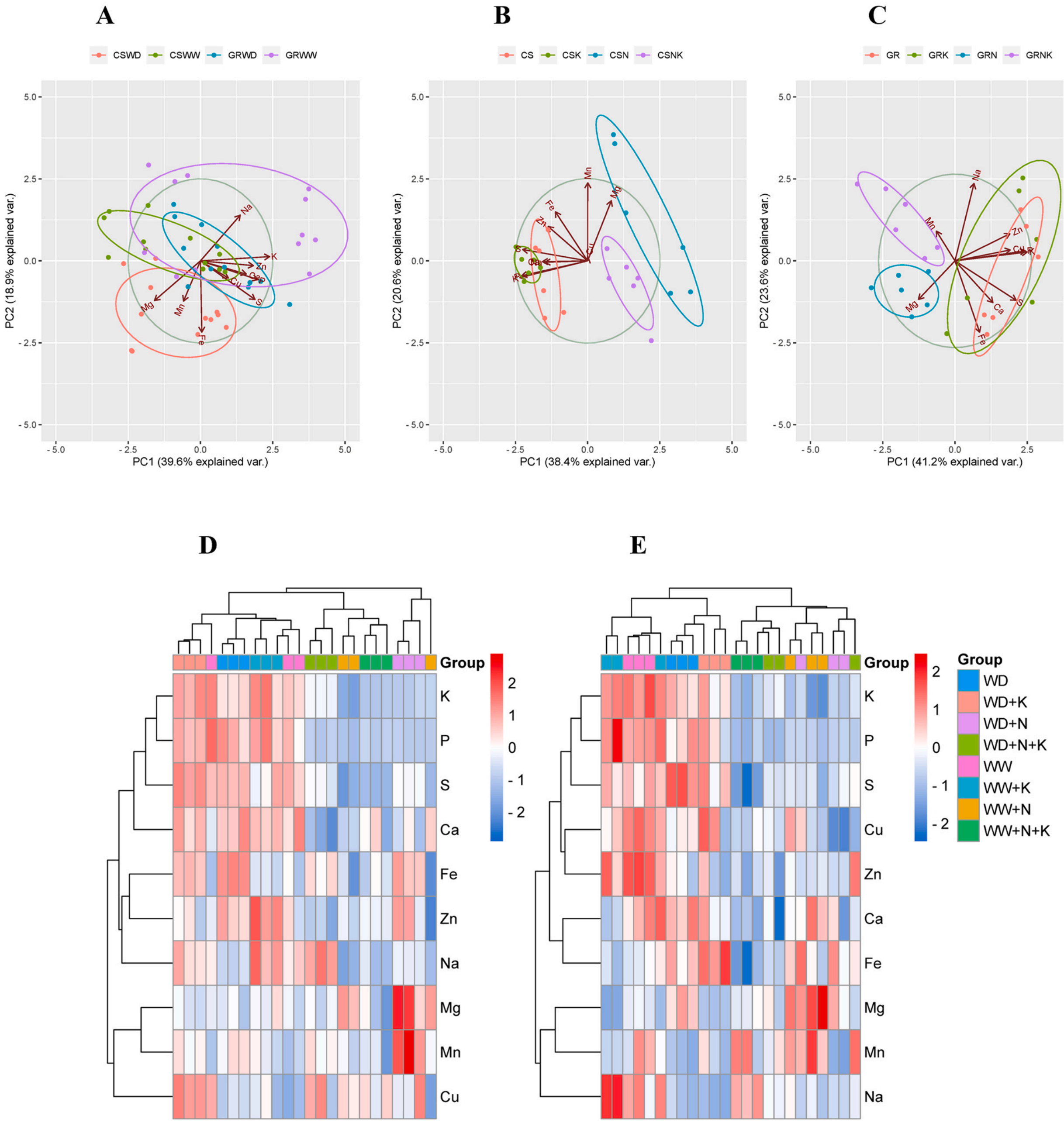

Fig. 6. Elemental concentration of grapevine leaves collected at maturity (S3). Principal component analyses (PCAs) of Cabernet Sauvignon and Grenache cultivars grouped by water treatment (**A;** CSWD or CSWW: Cabernet Sauvignon under 33 % ET_{lys} or 100 % ET_{lys}, respectively; GRWD or GRWW: Grenache under 33 % ET_{lys} or 100 % ET_{lys}, respectively; irrespective of N and K treatments), and Cabernet Sauvignon (**B**; CS: Cabernet Sauvignon under 30 % N + 30 % K; CSK: Cabernet Sauvignon under 30 % N + 100 % K; CSN: 'Cabernet Sauvignon' under 100 % N + 30 % K; CSNK: Cabernet Sauvignon under 100 % N + 100 % K, irrespective of water treatment) and 'Grenache' (**C**; GR: Grenache under 30 % N + 30 % K; GRK: Grenache under 30 % N + 100 % K; GRN: Grenache under 100 % N + 30 % K; GRNK: Grenache under 100 % N + 100 % K, irrespective of water treatment) samples grouped by fertilization treatment. Heatmaps show hierarchical clustering of elemental concentration of Cabernet Sauvignon (**D**) and Grenache (**E**) samples (N = 3). WD, water deficit 30 % N + 30 % K; WW, well-watered 30 % N + 30 % K; WD+K, water deficit 30 % N + 100 % K; WW+K, well-watered 30 % N + 100 % K; WD+N, water deficit 100 % N + 30 % K; WW+N, well-watered 100 % N + 30 % K; WD+N+K, water deficit 100 % N + 100 % K; WW+N+K, well-watered 100 % N + 100 % K.

low N supply, regardless of water treatment and cultivar, although this effect was more pronounced in 'Cabernet Sauvignon'. At veraison, K, P, and S were consistently higher under low N supply, especially under well-watered conditions. The highest P levels were found in WW plants in both cultivars, with significantly higher values under well-watered compared to water deficit conditions in 'Grenache' (Supplementary Table S8). At maturity, the effect of N treatment became even more pronounced, with K, P, S, and, to a lesser extent, Zn concentrations higher under low N levels. In 'Grenache', for example, WW+K and WW plants displayed significantly higher K concentrations (11 and 11.5 mg • g^{-1}) compared to WW+N+K and WW+N plants (5.4 and 4.2 mg • g^{-1}). Lowest Fe values were observed in +N+K 'Grenache' plants, although this was significant only under water deficit conditions (Supplementary Table S9). Few exceptions to this general trend can be observed, such as for N and Ca concentrations. As expected, at veraison, 'Cabernet Sauvignon' plants under high N supply (WW+N+K and WW+N) exhibited higher N concentration than plants under low N (WW+K and WW). On the other hand, at maturity, 'Grenache' plants under low N displayed higher N concentration (especially WW+K compared to WW+N+K). At veraison, higher Ca values are observed in WW+N compared to WW plants in 'Grenache', whereas at maturity, higher Ca levels are observed in WD+K compared to WD+N+K plants in 'Cabernet Sauvignon'.

K treatment significantly affected the concentration of several nutrients, both as single and in combination with other factors such as water and N treatments (Supplementary Table S6). At flowering, lower N levels are shown in 'Grenache' plants under high K compared to low K supply. At veraison, 'Cabernet Sauvignon' under well-watered conditions exhibited higher K concentrations under high K levels. Under water deficit conditions, lower Ca levels are observed in 'Cabernet Sauvignon' plants treated with both high N and K supply compared to single N and K treatments. Higher Fe concentrations are found in WW+N and WW compared to WW+N+K and WW+N. At maturity, Mg concentration reached its highest level under +N-K treatment, independent of water treatment and cultivar, whereas the high K treatment led to an increase in Na concentration in 'Cabernet Sauvignon' under water deficit conditions and in 'Grenache' under well-watered conditions.

4. Discussion

4.1. *Water deficit decreased shoot growth and gas exchange, but increased leaf nutrient concentrations*

Water availability plays a crucial role in vine growth, and it is well known that deficit irrigation inhibits leaf lamina and internode expansion, leading to a reduction of vegetative growth and pruning weight (Chaves et al., 2010; Lovisolo et al., 2010; Seleiman et al., 2021; Zamorano et al., 2021). In agreement with these findings, in the present experiment, water deficit significantly reduced vine vigor. In modern viticulture, the control of vegetative growth through partial water restriction is often deliberately applied, since Regulated Deficit Irrigation (RDI) strategies have been demonstrated to increase the skin-to-pulp ratio and enhance the concentration of terpenes, anthocyanins, and polyphenols in berries (Calderan et al., 2021; Sivilotti et al., 2024). Since similar assimilation rate (A) values were observed between treatments, the reduced growth in water deficit plants could be linked to altered biomass allocation rather than a limited carbon assimilation. In addition, previous studies have observed that vines under water deficit presented higher root-to-shoot ratios and root dry matter content, a strategy that could enhance drought tolerance (Simonneau et al., 2017; Vuerich et al., 2021).

Stem water potential (Ψ_{stem}) levels reflect both soil water availability and the evaporative demand of plants, being a common parameter used to describe plant water status (Choné et al., 2001). Ψ_{stem} values in grapevine usually range from −1.2 to −1.4 MPa when moderate to severe water deficit conditions are imposed (Dayer et al., 2019). In this experiment, although water deficit reduced vine Ψ_{stem}, values never dropped to critical thresholds and remained above −1.0 MPa, suggesting that 33 % ET_{lys} treatment conditions induced only a mild water stress in grapevine. Therefore, the physiological responses of the two cultivars may differ under a more severe water deficit, as plants may alter their hydraulic behavior depending on the intensity of the stress (Gambetta et al., 2020). Nevertheless, the present experiment allowed us to capture early physiological adjustments, while irreversible damage to plants was avoided.

Stomata closure is one of the earliest plant responses to water stress to avoid excessive water loss and prevent water potential from becoming too negative (Gambetta et al., 2020; Schultz et al., 2003). In agreement with previous findings (Bota et al., 2016; Poni et al., 2014; Torres et al., 2021), in this experiment, the g_s reduction under water deficit conditions implied lower water consumption, which consequently improved the intrinsic water use efficiency (iWUE) compared to well-watered plants. These results suggest that, since A was not significantly altered by water deficit, stomatal regulation could be considered as the main factor influencing iWUE.

Nevertheless, as shown by both Ψ_{stem} and gas exchange measurements, the effect of irrigation treatment was more pronounced at early developmental stages when grapevine plants are particularly sensitive to drought stress (Herrera et al., 2022; Król and Weidner, 2017). The prompt stomatal closure observed during early season prevented Ψ_{stem} from becoming excessively negative and causing xylem cavitation (Dayer et al., 2019; Hochberg et al., 2017b). Throughout the experiment, plants acclimatized well to water deficit, as shown by the progressive increase of g_s. This effect can be linked to the reduction of shoot growth that in turn enabled individual leaves to maintain a higher transpiration. Given that water deficit imposition was maintained throughout the season, chemical rather than hydraulic signals may have played a more prominent role in modulating stomatal activity (Tombesi et al., 2015). Overall, these results support the assumption that management practices based on RDI can maintain an acceptable plant water status and improve viticulture sustainability by optimizing water resources (Savi et al., 2018).

Most studies on the ionomic profile of grapevine have been focused on fruit nutritional quality, plant-pathogen responses, and Cu toxicity (for review see Savoi et al., 2022). However, little attention has been paid to the impact of water deficit on vine nutritional status. In the present experiment, water deficit at flowering increased the concentration of several nutrients in leaves (such as K, Mn, P, and S); vice versa, in well-watered plants the reduced elemental concentration might be a consequence of a "dilution effect", resulting from their increase in biomass accumulation in the vegetative part (Jarrell and Beverly, 1981; Trouvelot et al., 2015).

4.2. *N fertilization increased shoot growth and water use efficiency, but decreased leaf nutrient concentrations*

Nitrogen fertilization promoted shoot elongation under both water regimes (WW+N vs. WW and WD+N vs. WD), with the highest values recorded in WW+N plants. The role of N fertilization in promoting vegetative growth is well documented (Metay et al., 2014; Schreiner et al., 2013; Squeri et al., 2021; Zanin et al., 2022). However, promoting shoot growth under high N fertilization doses would increase plant water demand, subjecting the plants to possible water stress. Similar findings on the interaction of nutrient supply and drought stress were reported in apricot and grapevine studies, where enhanced fertilization increased vegetative growth but also aggravated drought sensitivity (Dogan et al., 2025; Daler et al., 2025a). Additionally, in agreement with recent viticultural research, sustainable vineyard management practices, such as soil amendments and optimized rootstock-scion combinations, have been shown to improve grape quality and resilience (Daler et al., 2025b; Kaya et al., 2025). In this experiment, high N supply led to a reduction in g_s (particularly in 'Cabernet Sauvignon'), improving iWUE. On the other hand, although high N supply resulted in higher vegetative vigor, no critical Ψ_{stem} values were recorded neither in 'Cabernet Sauvignon' nor in 'Grenache', and hydraulic failures were avoided even under high fertilization doses. These results are in line with the findings of Faralli et al. (2023), in which grapevine water status under optimal environmental conditions was linked to nitrate availability, although different rootstocks and root traits may further influence this response (Bernardo et al., 2025). Similarly, lower g_s rates were also recorded in various grapevine rootstocks subjected to higher N supply levels (Zamboni et al., 2016). However, the relationship between N fertilization and plant water status can vary depending on several factors, including cultivar, phenological stage, and N dosage, which can even lead to a reduction in iWUE under high N application (Taskos et al., 2020). This suggests complex interactions driven by N availability and reinforces the need to consider both water and nutrient management strategies for optimizing vine health and productivity.

Notably, while water deficit tended to increase leaf nutrient concentrations, higher N supply resulted in lower concentrations of key elements such as K, P, and S, likely due to the increased biomass growth under high N levels. In addition, since root nitrate uptake tends to induce an alkalinization of the rhizosphere pH, it is possible that the solubility and uptake of several micronutrients were reduced, along with the bioavailability of other elements such as P (Abel, 2017; Espen et al., 2004; Gómez-Suárez et al., 2020; Matsuoka et al., 2019). Recent findings have demonstrated that grapevine responses to Fe deficiency are highly influenced by the N form supplied, with NH_4^+ addition able to promote stronger Fe-acquisition mechanisms than NO_3^- (Khalil et al., 2024). Previous studies observed a similar decline in leaf nutrient concentrations when N is increased, albeit with genotypic variations associated with different rootstocks (Lecourt et al., 2015; Peuke, 2009; Schreiner et al., 2013; Zamboni et al., 2016).

4.3. K fertilization effects on leaf ionomic profile

In comparison to water and N treatments, K fertilization exerted smaller effects on morphological and physiological parameters. Unlike NO_3^-, which is primarily transported via mass-flow, a mechanism sensitive to soil moisture, K is predominantly transported through diffusion (Marschner, 2011), possibly making it less susceptible to fluctuations in water availability. Consequently, the differential movement mechanisms of these nutrients could explain the more pronounced effects of N compared to K under varying irrigation regimes. In addition, a significant portion of K required for fruit development is supplied through remobilization from reserves stored in the vine's permanent structures, whereas only 50 % or less is provided by root uptake from the soil (Conradie, 1981).

Nevertheless, the effect of K fertilization on the ionomic composition of grapevine leaves was evident. As expected, K concentrations increased under high K supply, particularly at veraison in 'Cabernet Sauvignon'. From the principal component analysis (PCA), a clear separation between K treatments was observed under high N supply (WW+N+K vs. WW+N and WD+N+K vs. WD+N) and started to be visible at veraison in 'Cabernet Sauvignon' and only at maturity in 'Grenache'. The combined N and K fertilization led to an overall increase in Na and reduction in Mg concentrations. It has been reported that Na, when present in small amounts, can exert beneficial effects as a "cheap" osmolyte (Raddatz et al., 2020). Since Na can be taken up by several K^+ transporters, its higher concentration in leaves may be related to the activation of K^+ non-specific acquisition systems in response to K fertilization. On the other hand, the antagonistic effect of K on Mg concentrations in leaves may reflect competitive effects among cations, whose uptake by plants is influenced by their availability in the rhizosphere (Xie et al., 2021).

Interestingly, at veraison, K fertilization applied in combination with N (WW+N+K vs. WW+N) led to a significant reduction in Fe concentration in leaves. K fertilization has been reported to stimulate NO_3^- uptake (Coskun et al., 2017 and references therein; Raddatz et al., 2020), which may lead to a reduction in Fe bioavailability in the rhizosphere via soil alkalinization induced by root-mediated nitrate acquisition.

4.4. Cultivar-specific responses to the combined water x N x K treatments across different developmental stages

Grapevine physiological responses to water deficit markedly vary with scion genotype (Bota et al., 2016; Prieto et al., 2010; Serrano et al., 2022), rootstock (Tramontini et al., 2013), and even clone (Tortosa et al., 2016). Based on their stomatal sensitivity to soil drying, 'Grenache' is often classified as a near-isohydric cultivar, whereas 'Cabernet Sauvignon' as a near-anisohydric one (Keller, 2020; Lovisolo et al., 2010; Schultz, 1995; Schultz, 2003). In this study, the two cultivars exhibited distinct response patterns under the combined water and N treatments, likely due to their different hydraulic strategies. However, although 'Cabernet Sauvignon' exhibited greater vegetative growth, 'Grenache''s typical conservative behavior did not translate into a significantly higher stomatal control under water deficit conditions, as both cultivars experienced similar Ψ_{stem} declines. Indeed, different genotypes may change their stomatal behavior based on environmental factors and on the severity and intensity of stress (Gambetta et al., 2020). Potted cuttings of 'Grenache' subjected to fast or slow-developing drought switched their hydric strategy depending on the progression of water stress (Morabito et al., 2022). Additionally, grapevine resilience to water stress has been shown to increase over the course of the growing season (Herrera et al., 2022). Based on these observations, the distinction between isohydric and anisohydric behavior should not be decisive, given that both strategies may manifest during the season and across different environmental contexts (Lavoie-Lamoureux et al., 2017).

In the present study, regarding physiological measurements, 'Cabernet Sauvignon' exhibited a stronger response to N treatment, and a seasonal shift in its sensitivity to water and N treatments. Early in the season, 'Cabernet Sauvignon' showed a marked reduction in g_s under water deficit, thereby improving iWUE. However, later in the season, and particularly from July onwards, N treatment played a dominant role in modulating vine water status, enhancing iWUE. Conversely, although both cultivars showed similar Ψ_{stem} reductions, 'Grenache''s physiological response was consistently modulated by water availability throughout the season, with no observed N effects on gas exchange, in line with its more conservative water-use tendency. The different responses of 'Cabernet Sauvignon' and 'Grenache' to N treatment may be linked to cultivar-specific anatomical and physiological traits. These cultivar-dependent responses are consistent with previous reports that highlighted the modulation of stress-tolerance mechanisms in grapevines under abiotic stress, including temperature fluctuations and soil-induced lime stress (Daler et al., 2025b; Kaya et al., 2024). In addition to physiological adjustments, differences in nutrient acquisition strategies among varying scions and rootstocks have been reported, such as the activation of Fe-deficiency responses under variable N forms or alkaline soils, further illustrating the complexity of genotype-dependent resilience mechanisms (Khalil et al., 2024, 2025). Although 'Grenache' is generally classified as isohydric, studies have reported that it could sustain high hydraulic conductivity due to its larger vessel diameters (Shelden et al., 2017; Tombesi et al., 2014), higher stomatal density (Gerzon et al., 2015) and osmolyte accumulation (Tamayo et al., 2023). In this experiment, however, the increased vigor observed in 'Cabernet Sauvignon' under high N supply is likely associated with increased canopy transpiration demand. Consequently, the reduction in g_s observed under high N in 'Cabernet Sauvignon', and not in 'Grenache', may reflect a stronger need for stomatal regulation to limit excessive water loss associated with its vigorous canopy development.

Similar to the physiological response, water and N treatments influenced leaf elemental concentrations in a developmental stage-dependent manner and depending on the scion genotype. At flowering, the response was mainly modulated by water treatment, whereas starting from veraison, N treatment emerged as the dominant factor, followed by the K one. The less pronounced effect of N and K treatments at flowering may reflect plant reliance on nutritional reserves accumulated in previous seasons and stored within woody tissues (Pradubsuk and Davenport, 2010; Zufferey et al., 2015). On the contrary, after flowering, grapevine usually exhibits a higher capacity for N uptake from soil (Holzapfel et al., 2019; Zapata et al., 2004). A first N uptake peak is observed only after flowering (Holzapfel et al., 2019), followed by a smaller peak at the end of the growing season (Keller, 2020; Zanin et al., 2022). Thus, the timing of N fertilization is critical for optimizing nutrient use efficiency and ensuring sustainability in viticulture. Moreover, the ionomic composition of leaves indicated cultivar-specific seasonal variations. While 'Cabernet Sauvignon' showed a clear differentiation based on N treatment (and, to a lesser extent, K treatment) already at veraison, in 'Grenache' this distinction became more evident only at maturity. The delayed response of 'Grenache' in the multielemental composition of leaves may reflect its slower vegetative growth rate, being less affected by N (or K) regimes. This response can be related to a higher resilience of 'Grenache' compared to 'Cabernet Sauvignon', which allows 'Grenache' to adjust its nutritional status being less dependent on environmental fluctuations (in this case nutrient availability).

5. Conclusions

This work provided evidence of the intricate, developmental-stage-dependent interplay between water and N x K treatments in shaping vine growth, physiology, and nutrient allocation. 'Cabernet Sauvignon''s anisohydric nature allowed for a more dynamic response to N fertilization, while 'Grenache''s isohydric tendency resulted in a more stable physiological and nutritional status. These findings highlight the importance of managing irrigation and fertilization practices together, in the framework of a sustainable viticulture under the challenges of climate change. Nevertheless, future experiments under field conditions will be crucial to confirm the observed nutritional and varietal patterns under the complexity of vineyard edaphic and climatic conditions.

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CRediT authorship contribution statement

Gabriella Vinci: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Alberto Calderan:** Writing – review & editing, Investigation. **Arianna Lodovici:** Writing – review & editing, Investigation. **Marianna Fasoli:** Writing – review & editing, Supervision, Funding acquisition, Data curation, Conceptualization. **Paolo Sivilotti:** Writing – review & editing, Supervision, Data curation, Conceptualization. **Laura Zanin:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.envexpbot.2025.106294.

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