



ORIGINAL RESEARCH ARTICLE

# Impact of contrasting soils in a high-altitude vineyard of *Vitis vinifera* L. cv. Malbec: root morphology and distribution, vegetative and reproductive expressions, and berry skin phenolics

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## ABSTRACT

The high-altitude viticulture of Mendoza is being increasingly recognised among the top wine regions worldwide. High levels of solar ultraviolet-B (UV-B) radiation, alluvial heterogeneous soils and cool night temperatures are major influences on *Vitis vinifera* L. phenotypic characteristics, especially on Malbec, Argentina's emblematic grapevine variety. In the present work, the effect of intra-vineyard contrasting soil depth on Malbec was evaluated in a high-altitude vineyard (1450 m a.s.l.), over two growing seasons for physiological traits and over three vintages for yield components and berry skin phenolic compounds. The experiment was carried out in two parcels of shallow (SS) and deep (DS) soils, with different physico-chemical characteristics, and submitted to equal irrigation and crop management. Results showed that root depth was not limited by any soil physical constraints and fine roots (< 1 mm) were patchily distributed in sites with higher contents of silt, clay and organic matter in SS. Higher root quantity in SS was related to lower vegetative and reproductive expressions, shorter ripening period and reduced berry size and fruit yield when compared to DS. When SS plants experienced mild water stress ( $\Psi_s$ , stem water potential, between -6 and -9 bar) increments of anthocyanins, total polyphenols and antioxidant capacity in berry skins were observed. However, when SS plants reached  $\Psi_s \leq -10$  bar, physiological harm occurred. The results presented contribute to understanding the effects of soil type on Malbec vines during different seasonal environmental conditions, especially in stressful situations, such as those expected by climate change scenarios.

## KEYWORDS:

berry quality, grapevine, intra-vineyard variability, root architecture, terroir, vintage effect, water stress

## INTRODUCTION

Wine quality depends mainly on the specific site where vines grow; that is, a combination of soil, climate, plant material and cultural techniques, which is known as *terroir* (Seguin, 1986; van Leeuwen and Seguin, 2006). Over the last decades the Mendoza (Argentina) wine industry has explored new viticulture areas, even those located at elevations as high as 1500 m a.s.l., where red grapevine cultivars show premium oenological qualities. In general terms, this is attributable to higher daily temperature amplitude caused by cooler nights and increased solar ultraviolet-B (UV-B) radiation, as compared to vineyards located at lower elevations (Berli *et al.*, 2010). Soil heterogeneity is another characteristic of the high-altitude vineyards in the Andes foothills of Mendoza. Due to their alluvial origins, these Entisols (unstructured soils) vary in terms of the volume of soil explorable by roots (Zárate and Mehl, 2019), called effective soil depth, which plays a key role in *terroir* expression. Soil characteristics have a major influence on plant performance and, therefore, on viticultural approaches to managing specific sites inside the vineyard (Reynolds *et al.*, 2013; Reynolds and Rezaei, 2014; Brillante *et al.*, 2016). This spatial variation determines the differences in phenology, vegetative balance (Gatti *et al.*, 2022; Verdugo Vazquez *et al.*, 2022), berry growth and wine quality (van Leeuwen *et al.*, 2004; Morlat and Bodin, 2006; Priori *et al.*, 2019; Sams *et al.*, 2022). Most research on this topic has been conducted in regions where drainage is needed to moderate the excessive water supplied by rainfall to prevent waterlogging and, thus oxygen starvation, in the root zone (de Andrés-de Prado *et al.*, 2007; van Leeuwen *et al.*, 2009; Tomasi *et al.*, 2015; Bordoni *et al.*, 2016). Under such conditions, shallow soils are generally associated with potential sites for premium wines, providing limited vegetative growth and fruit yield (van Leeuwen *et al.*, 2004; Coipel *et al.*, 2006; Morlat and Bodin, 2006; de Andrés-de Prado *et al.*, 2007). However, renowned wines from all over the world come from different soil types, and information on how they modulate grapevine physiology, especially in high-altitude vineyards, is limited. It has been suggested that a specific *terroir* can be explained by the soil's physical properties, including cover crops that affect root development and water supply regulation (Tomasi *et al.*, 2015; van Leeuwen and De Ressaiguier, 2018; Fleishman *et al.*, 2019). This concept is particularly relevant in arid regions such as Mendoza (250 mm of average annual rainfall), where irrigation is mandatory for viticulture. Shallow soils are generally associated with superficial root systems, which are prone to suffer water stress events (Tomasi *et al.*, 2015). Notwithstanding, the bedrock can be artificially broken by trenching, which allows the roots to penetrate the rock cracks at depth (Smart *et al.*, 2006; Savi *et al.*, 2019; Costantini, 2021). Root distribution throughout the soil profile is not homogeneous due to patches with differential nutrient and water availability (Smart *et al.*, 2006; Karakis *et al.*, 2017). Recently, cloddiness in the soil profile was considered, affecting estimations of plant rooting capacity (Priori *et al.*, 2020; Costantini, 2021). In the heterogeneous soils of Mendoza's Andes foothills, soil spatial variability

with considerable depth differences is common and may influence grapevine performance, as well as root distribution.

High UV-B radiation level has been shown to increase secondary antioxidant metabolites in berry skins, also affecting vegetative growth and fruit yield (Berli *et al.*, 2013; Berli *et al.*, 2015). Moreover, high levels of UV-B, especially when combined with other abiotic stresses such as moderate water stress, may cause a more prominent reduction in chlorophyll photosynthetic pigments and an increase in the accumulation of berry skin polyphenols, although with a reduction in yield, implying more severe stress conditions than if exposed to single abiotic stressors (Berli *et al.*, 2015; Alonso *et al.*, 2016; Doupis *et al.*, 2020). Among other stressful factors, air temperatures above 35 °C decrease the biosynthesis of secondary metabolites (Mori *et al.*, 2007; Zarrouk *et al.*, 2016), as well as photosynthesis rate and vegetative growth (Galat Giorgi *et al.*, 2019). In the Mendoza foothills, the complex interaction of the prevailing abiotic factors along with heterogeneous soils may represent an unexplored influence on grapevine physiology.

In Argentina, 70,4 % of the total area planted with grapevines is concentrated in Mendoza and 84,6 % of the grapevines are Malbec (INV, 2021). This cultivar presents high phenotypic plasticity; i.e., the ability to express different phenotypes as a function of the environment (Marfil *et al.*, 2019), including soil heterogeneity (Roig-Puscama *et al.*, 2021).

Therefore, the current Malbec typicity could be altered in a context of climate change, where an increase in air temperatures, more frequent and longer duration of heat waves, as well as a decrease in water availability for irrigation, are expected in future years (IPCC, 2021). Moreover, there is a projected displacement of Argentinean wine-growing regions to higher elevations by the end of this century, as suggested by climatic model simulations (Cabr   and Nu  ez, 2020). Thus, information about the influence of soil type, sunlight quality and air temperature, as *terroir* modelling factors for Malbec in high-altitude viticulture, is a present-day matter of interest.

In this study, the vegetative and reproductive expression and root distribution of Malbec in a high-altitude vineyard were analysed within vineyard contrasting soil types (same irrigation and crop management) over two growing seasons for plant physiology and over three vintages for yield components and berry skin phenolics. A better understanding of these interactions is crucial for an adequate decision-making in the viticulture industry.

## MATERIALS AND METHODS

### 1. Site selection, plant material and experimental design

The experiment was carried out on the 2016, 2017 and 2018 vintages and during the 2016-2017 (2017) and 2017-2018 (2018) growing seasons, in a high-altitude commercial vineyard (1450 m a.s.l.) located in Gualtallary (69°15'W and 33°23'S), Mendoza, Argentina.

The formation of the soil in the area occurred on Quaternary sedimentary deposits from alluvial fans which had developed at the foot of the Andes mountains. The place of study is characterised by superficial fluvial and aeolian sedimentary deposits that have accumulated since the middle Pleistocene (Polanski, 1963; Pépin *et al.*, 2013). Erosion and sedimentation phases shaped the landscape, and soil formation determined soils formed by sandy-silts deposits with parent materials of volcanic and metamorphic lithologies (Figure 1 A; Mehl and Zárate, 2012). Figure 1 B shows that the dominant soil order of the study site is the Entisols and the suborder is typic Torriorthent (Regairaz *et al.*, 1996); i.e., mineral soils with a non-differentiated profile and scarce or no pedogenic development due to a short-term evolution under an arid (or torric) soil moisture regime (Abraham *et al.*, 1996; Pérez Valenzuela, 1999; Rubio *et al.*, 2019). The genesis of the Gualtallary alluvial fan is related to the alluvial fan upstream of Las Tunas River. The Gualtallary fan developed flanking the El Jaboncillo and El Peral hill, two units of the landscape uplifted as a result of the Andean tectonic influence in the piedmont area (Figure 1 C; Pepin, 2010; Corona, 2019).

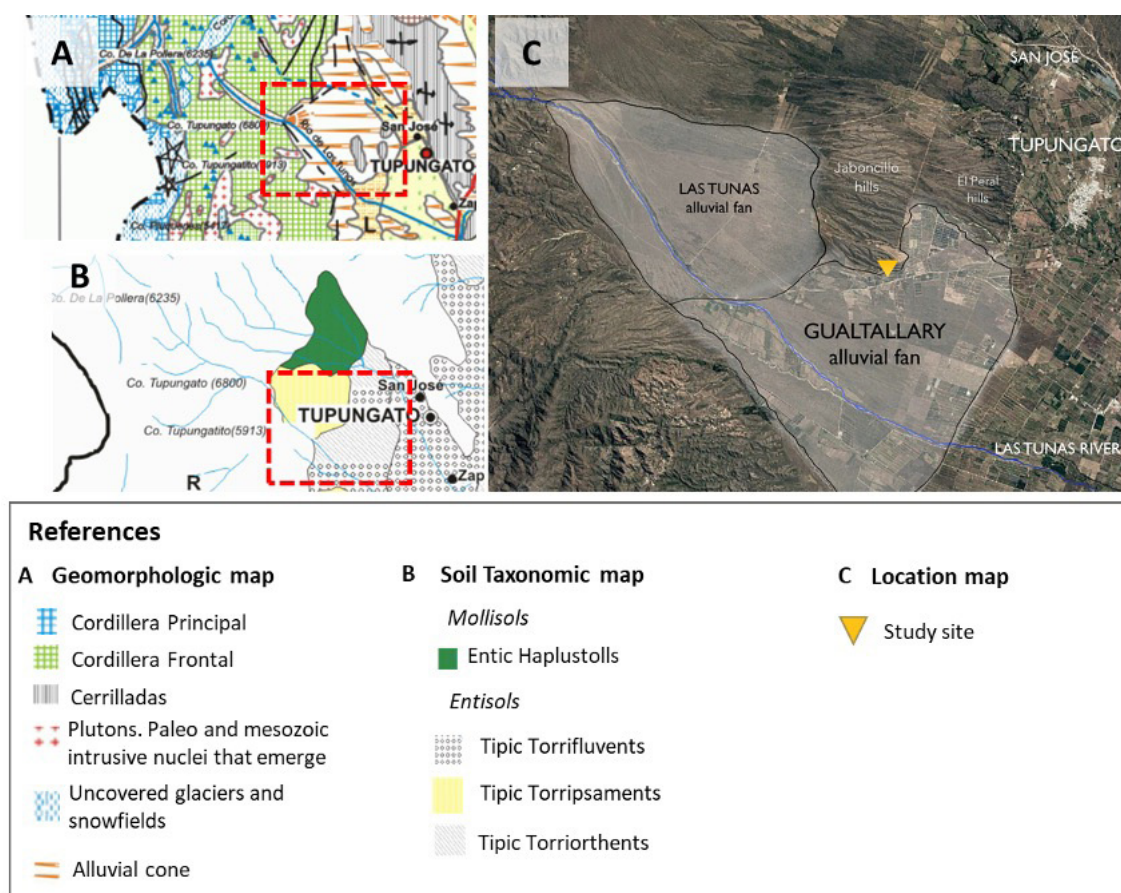
The 23-year-old own-rooted Malbec vines were trained in a vertical shoot positioning trellis system (2 m x 1.20 m; double Guyot uniformly pruned), and hail protected (nets). The vineyard was drip-irrigated with pressure compensating emitters spaced at 0.65 m delivering 2.2 L/h. The trial was

carried out on two contrasting soils located in the same vineyard in plots with an area of 0.15 ha each and 30 m apart on a slope of 5.4 %. Based on the effective soil depth, soils were identified as deep soil (DS: 1 to 1.5 m depth, foot slope position) and very shallow and shallow soil (SS: 0 to 0.45 m depth, top slope position, named shallow) according to classes of root-restricting depth proposed by NCRS Soils (usda.gov). Their taxonomic classification, physicochemical composition and location in the vineyard are presented in Supplementary Material as Tables 1S, 2S and 3S, and Figure 1S respectively. Soil analyses and the experimental plot selection methodology are fully detailed in the Supplementary Material section. Both SS and DS were classified as sandy, according to the International System of the Soil Science Society classification (Gee and Or, 2002).

The experiment was carried out as a split-plot design (SS and DS) with 12 randomly chosen experimental units as replicates, each one composed of 6 plants.

## 2. Environmental data

The average air thermal values (daily mean, maximum and minimum) and rainfall were registered in the vineyard from September to March (2015-2018) by a weather station PEGASUS EP201 (TECMES Instrumentos Especiales SRL, CABA, Argentina). Recordings of the mean monthly air temperature (MMAT; °C) and the mean monthly air relative



**FIGURE 1.** Geomorphological context of the site of study. A. Geomorphologic map B. Taxonomic map and C. Location map. Adapted from Abraham *et al.* (1996); Regairaz *et al.* (1996) and Corona (2019).



humidity (MMRH; %) at the bunch level in SS and DS sites, were performed by the i-Button Hygrochron 1923DS sensors (Maxim Integrated Products Inc., CA, USA) protected by a perforated polyethylene cap over the same period of time for the growing seasons 2017 and 2018 ( $n = 3$  in 2017,  $n = 4$  in 2018). In 2018, the mean monthly soil temperature (MMST) from September to March was calculated in SS and DS from recordings by the DECAGON MPS-6 dielectric sensors (Decagon Devices Inc., WA, USA) located at depths of 0.3, 0.6, 0.9 and 1.2 m. Maximum solar ultraviolet-B ( $\mu\text{W cm}^{-2}$ ) and photosynthetically active radiation (PAR;  $\mu\text{mol m}^{-2}\text{s}^{-1}$ ) were measured at bunch level with a single day measurement (February 2018, post veraison stage) using a radiometer placed perpendicular to the sun PMA2200, a UV-B PMA 2102 erythral-weighted UV-B detector (Solar Light Co. Inc., PA, USA) and a light meter LI-250 with a quantum sensor LI-190SA (Licor Inc., NE, USA). Values were recorded every 60 min between 09:00 and 16:00.

### 3. Phenology, growth and yield-related variables

Phenology was monitored, as proposed by Coombe (1995), on 12 sentinel vines per soil type. In 2017 and 2018, at veraison, relative chlorophyll content (RCC), leaf area (LA) and maximum quantum yield of PSII (Fv/Fm) were determined. Twenty minutes of dark adaptation with a leaf clip was used to allow various photosynthetic and photoprotective mechanisms and state transitions to relax. The variables were measured on randomly selected plants (one per experimental unit;  $n = 12$ ). A SPAD 502 (Konica Minolta, Osaka, Japan) was used to calculate RCC as an average measure from the third to fifth basal leaves on two selected shoots per plant. The LA per plant was estimated as the mean value of odd leaves from two shoots per plant, multiplied by the number of shoots, and determined through digital images (Easy Leaf Area software; Easlon and Bloom, 2014). To represent canopy architecture, LA along odd leaves per shoot for SS and DS vines was quantified at veraison of the 2017 and 2018 seasons ( $n = 24$ ). The Fv/Fm ratio was measured on one randomly selected, fully expanded East facing leaf per plant, from the middle section of the canopy, using a chlorophyll fluorometer (Handy Pocket PEA, Hansatech Instruments Ltd., Norfolk, England). The stem water potential ( $\Psi_s$ ) was determined at midday from December to March during seasons 2017 and 2018 with a frequency of 10 to 15 days using the pressure chamber method (Williams and Araujo, 2002). Briefly, East facing adult leaves located in the middle section of the canopy were measured and water deficit characterisation was defined according to van Leeuwen *et al.* (2009).

The maturity level of berries was monitored by measuring soluble solids (SS; °Brix) with a Pocket PAL-1 digital hand-held refractometer (Atago Co. Ltd., Tokyo, Japan). At harvest, when berries had reached approx. 24 °Brix (range of differences between vintages and harvest dates are shown in Table 4S), the fruit yield per plant, number of bunches per plant, and average bunch fresh weight (FW) were analysed. In addition, the number of berries per bunch and berry FW was determined as the average of 4 bunches per plant.

In winter, the total number of shoots per plant, the shoot length and the weight of the pruning per plant were measured. The ratio between yield and pruning weight was expressed as Ravaz index.

### 4. Berry phenolic accumulation and antioxidant capacity

At 14, 27, 38 and 49 days after veraison (DAV), 10 berries per experimental unit were randomly collected from the middle, top and bottom parts of bunches for the following parameters: soluble solids (SS), berry skin total anthocyanins (TA) and total polyphenols (TP) according to Berli *et al.* (2008). At harvest, berry skin oxygen radical absorbance capacity (ORAC) was evaluated according to Berli *et al.* (2015) with a microplate fluorometer (Fluoroskan Ascent FL; Thermo Fisher Scientific, NC, USA).

### 5. Root morphology and distribution

During the winter of 2019, roots were evaluated by using the trench method (Bohm, 1979) in three pits per soil type. With the vine located at the centre, the grid (1 m x 1.50 m external frame and subdivisions of 0.1 m x 0.1 m) was placed 0.1 m from the trunk and 0.15 m from the ground. Three root-size categories were established based on diameter (Tomasí *et al.*, 2015): fine roots (< 1 mm), medium roots (1–2 mm), and woody roots (> 2 mm). The root density of each size category was calculated as the number of roots per square meter. The root distribution throughout the soil profile was represented and analysed as root density at depths of 0.45, 0.75, 1.05, 1.35 and 1.65 m. The relationship between fine roots (mainly used for vine nutrition and water uptake; Morlat and Jacquet, 1993) and soil physico-chemical properties was tested, since *in situ* observations showed that these roots were patchily distributed in the soil profile. Data of fine root density distribution are presented in Figures 3S and 4S (Surfer ® v. 17.1; Golden Software, CO, USA); fine root density values above the 90th percentile of data distribution were considered as high-fine root density, while values below the 10th percentile as low-fine root density. Then, patches of high- and low-fine root densities were identified throughout the soil profile. On each of these identified patches, soil samples were collected and mixed to gather one composed sample (1 kg) per pit for particle-size distribution and organic matter content evaluation.

### 6. Statistical analysis

Multifactorial ANOVA was applied after the evaluation of normality and homoscedasticity. The number of berries per bunch and pruning weight per plant were transformed by the square root to normalise the data set. The effects of soil type, season and their interaction were analysed on Fv/Fm, LA, RCC,  $\Psi_s$ , yield per plant, number of bunches per plant, number of berries per bunch, bunch FW, pruning weight per plant, and shoot length. The effect of soil type was studied for MMAT, MMST and MMHR. The effects of soil depth, soil type, and their interaction on root density and distribution were analysed. For TA, TP, and ORAC analyses, the effect of soil type, phenology (as DAV), season, and their interaction were tested. Media differences were analysed using the LSD

Fisher test ( $p < 0.05$ ). The relationship between high- and low-fine root density and soil physico-chemical parameters (organic matter, clay, silt and sand %) was analysed by a principal component analysis (PCA) using standardised data. Statistical analyses were performed with InfoStat v. 2017 software (Di Rienzo *et al.*, 2014). Only significant results are discussed.

## RESULTS

### 1. Environmental data

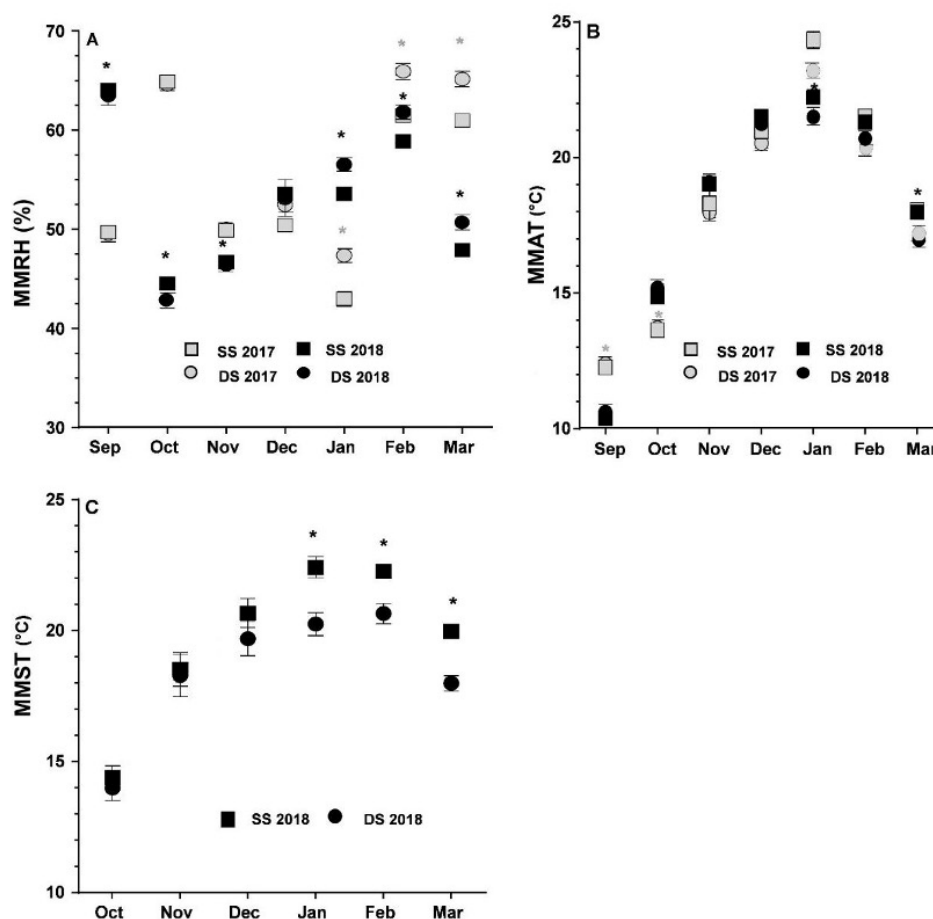
The 2016 season was the coldest and rainiest of the three vintages under study (Table 3S). Regarding vintages 2017 and 2018, the major difference was the amount of rainfall, which was 53 % higher in 2017 than in 2018. Mean monthly relative humidity values were higher in DS than in SS vines (Figure 2 A) from December to March (2017) and from January to March (2018). In January (both years), the temperature at bunch level was higher in SS than in DS (Figure 2 B). Mean monthly soil temperature was higher in

SS than in DS from January to March in 2018 (Figure 2 C). Based on the single day measurement of UV-B levels at bunch level, the SS plants were exposed to higher irradiances (3.6 times) than those of DS plants, especially during morning hours (Figure 3 A), coincidental with an increased (5.3 times) of PAR fluence rate (Figure 3 B).

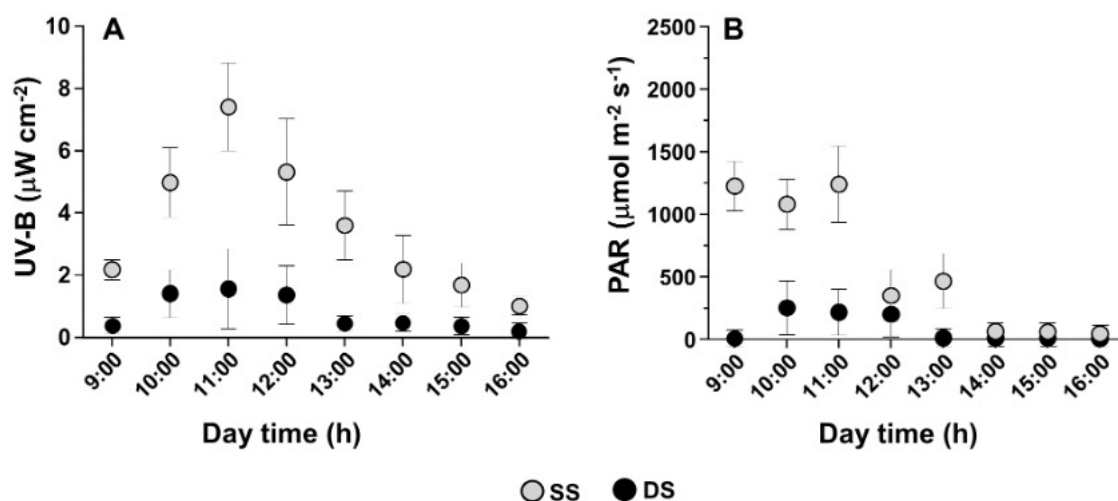
### 2. Phenology, growth and yield-related variables

Regardless of the season, the veraison to harvest period was shorter in SS plants than in DS plants. Also, in both soils, plants had a longer budbreak to full bloom period in 2017 than in 2018. In 2017, this phase started around 17 days before 2018, but finished at the same time. The number of days from full bloom to veraison remained unaltered (Figure 4). Harvest date occurred earlier for SS vines than for DS vines in seasons 2016, 2017 and 2018, as shown in Figure 4 and Table 4S.

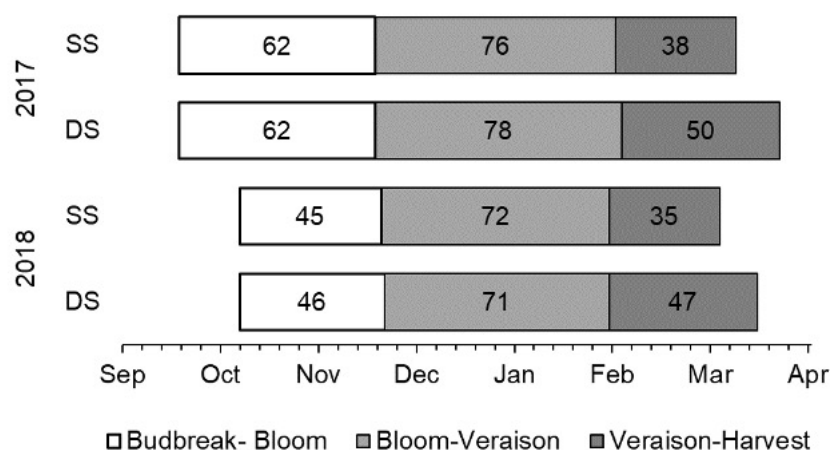
Leaves presented 27 % higher RCC in DS than in SS (Figure 5). Regarding Fv/Fm ratio, 2017 showed lower values in SS than the rest of the soil-year combinations.



**FIGURE 2.** Mean monthly air relative humidity (MMRH; A), mean monthly air temperature (MMAT; B), and mean monthly soil temperature (MMST, C), in shallow (SS; squares) and deep soils (DS; circles). Values are means  $\pm$  SEM. In A and B, grey symbols represent 2017 and black 2018 values. Grey and black asterisks represent  $p$  (soil)  $< 0.05$  within the same month for 2017 and 2018 respectively (LSD Fisher; A and B:  $n = 3$  in 2017 and  $n = 4$  in 2018; and C:  $n = 4$ ).



**FIGURE 3.** Diurnal variation of UV-B irradiance ( $\mu\text{W cm}^{-2}$ , A) and PAR fluence rate ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ , B) registered during a representative sunny post-veraison day at bunch level in shallow (SS) and deep soils (DS) vines during 2018. Values are means  $\pm$  SEM ( $n = 3$ ).



**FIGURE 4.** Phenological stages occurrence and duration in number of days for SS and DS grapevines (seasons 2017 and 2018). Each stage represents 50 % of the clusters reaching the following periods: budburst, bloom, veraison, and harvest.

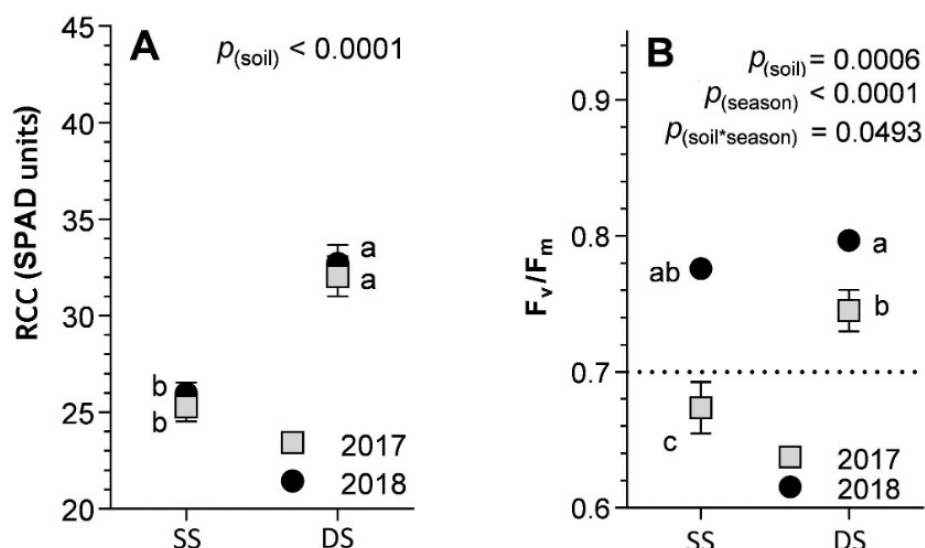
Regarding plant water status (Figure 6), in 2017, vines in DS experienced a weak water deficit from February to March (veraison to harvest), while those growing in SS experienced an anticipated and extended water deficit from January to February (post fruitset to post veraison) and a moderate to severe water deficit in March (until harvest). In 2018, in general, both SS and DS plants remained within weak water deficit levels.

The yield was 91 % higher in DS than in SS ( $p_{\text{soil}} < 0.0001$ ; Table 1). The number of bunches per plant, bunches FW and number of berries per bunch were higher in DS than in SS. Regarding growth parameters, average shoot length (150 %), as well as prune weight (244 %), number of shoots (30 %) and leaf area (150 %) per plant, were also higher in DS, compared to SS vines. Differences in canopy architecture can be observed in Figure 2S. The Ravaz index reveals an imbalance in SS vines respect to fruit production, influenced by season 2018. A seasonal effect was also observed on these variables, except for the number of berries per bunch

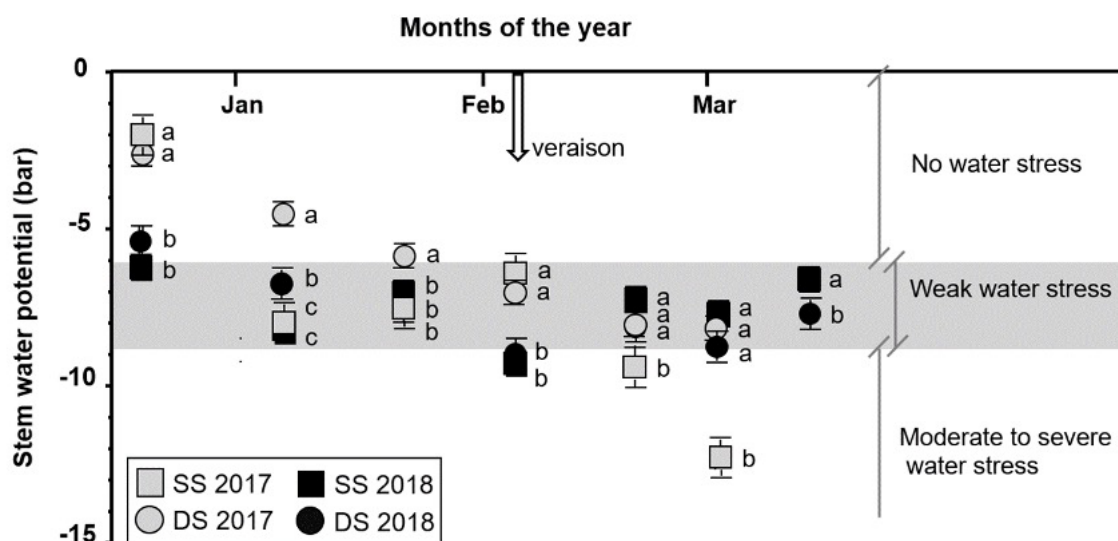
and shoot length (Table 1). On average, prune weight per plant was 45 % heavier in 2016 than in 2017 and 2018. In 2017, yield per plant was reduced by about 100 % compared to 2016 and 2018. There were about 10 more bunches per plant in 2018 than in the other seasons, and in 2016, bunches FW were approximately 33 g heavier than in the following seasons. There was no interaction between soil type and season for any other variable than berries FW and Ravaz index.

### 1. Berry phenolic accumulation and antioxidant capacity

A triple factor interaction effect (soil type, season and phenological moment) was observed for TA and TP at maturity (Figure 7 A and B). At 14 and 27 DAV in both seasons, SS berries showed higher TA and TP values than those of DS. In 2017 at 38 DAV, both TA and TP values in the SS berries were not only significantly lower than any other season-soil type combination, but they also experienced a drop (TA ~ 75 % and TP ~ 51 %) as compared to the previous



**FIGURE 5.** Multifactorial ANOVA of relative chlorophyll content (RCC; A) and maximum photosystem efficiency ( $F_v/F_m$ ; B) in SS and DS leaves from 2017 and 2018. (A) Mean values  $\pm$  SEM of 24 replicates. (B) Mean values  $\pm$  SEM of 12 replicates. Different letters show statistical differences (LSD Fisher;  $p < 0.05$ ).  $p(\text{soil})$ ,  $p(\text{year})$ ,  $p(\text{soil} \times \text{year})$ : effect of soil, season, and their interaction, respectively.



**FIGURE 6.** Stem water potential ( $\Psi_s$ ) of vines growing in shallow (SS, squares) and deep soils (DS, circles) from December to March (2017 and 2018 seasons). Values are means (grey for 2017 and black for 2018)  $\pm$  SEM ( $n = 12$ ) and different letters show statistic differences within each measure date (LSD Fisher;  $p < 0.05$ ).

date, while the rest of the combinations either increased or maintained their values. At harvest of DS vines, TA content decreased compared to the previous sampling date and, in 2018, these compounds were higher than in 2017.

Total anthocyanin, TP, and ORAC were higher in SS vine berries than in those of DS at harvest in 2016 and 2018 (Figure 8). During 2017, berries from SS plants had more ORAC but less TP than those of DS, while no differences were found for TA.

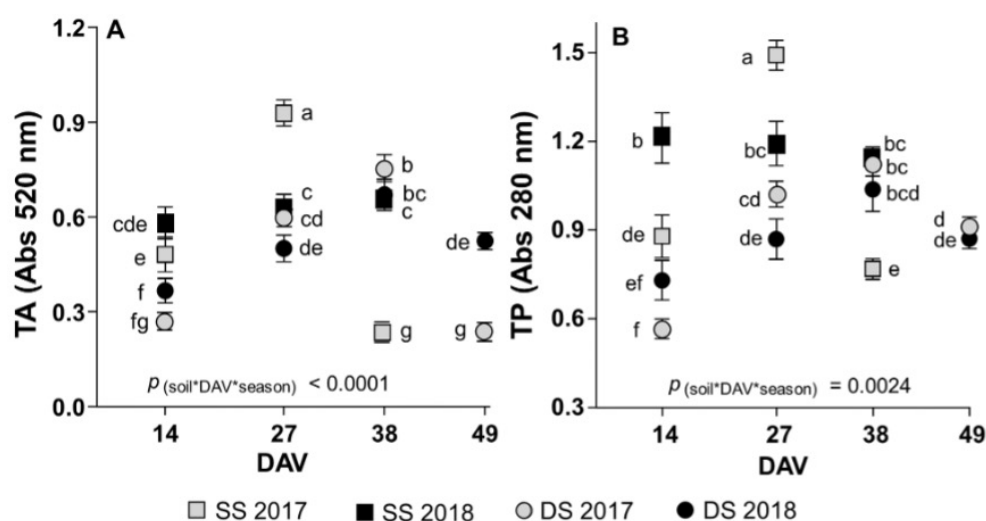
## 2. Root morphology and distribution

All root size categories were found up to a depth of 1.65 m in both types of soils and there was a significant interaction effect of soil type and depth on root distribution (Figure 9). There were 15 times more fine, medium and woody roots in the first 0.45 m of SS than in any other soil-depth combination. From 0.75 m downward there was no difference in root density of any size category between soil types. The number of fine roots across the entire grid was 4 times higher in SS than in DS vines (SS:  $4060.7 \pm 240$ ;

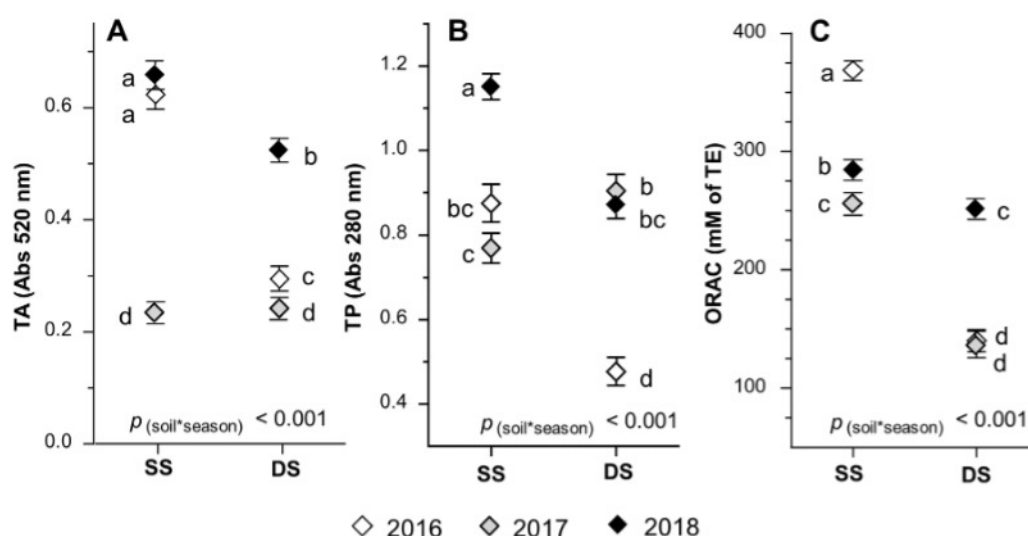
**TABLE 1.** Multifactorial ANOVA of yield components and vegetative expression of plants growing in shallow (SS) and deep soils (DS) (2016, 2017 and 2018 seasons). Values are means (n = 12) and different letters show significant differences (LSD Fisher; p < 0.05).

	Yield per plant (kg)	Clusters per plant	Cluster FW (g)	Berries per cluster	Berry FW (mg)	Prune weight per plant (g)	Shoots per plant	Shoot length (cm)	LA per plant (m2)	Ravaz index										
SS	1.07	b	22.6	b	52.9	b	47.3	b	1481	b	260.1	b	17.1	b	35.8	b	2.0	b	4.68	a
DS	2.10	a	29.0	a	80.4	a	58.3	a	1741	a	883.6	a	22.2	a	89.0	a	5.0	a	2.71	b
2016	1.99	a	21.8	b	88.9	a	49.6	a	2094	a	656.1	a	18.5	b	67.6	a	—	—	3.31	b
2017	0.84	b	23.0	b	64.5	b	51.1	a	1213	c	448.9	b	18.2	b	64.9	a	3.5	a	2.24	b
2018	1.92	a	32.5	a	46.5	c	57.8	a	1529	b	462.4	b	22.1	a	54.8	a	3.5	a	5.55	a
SS : 2016	1.30		17.8		69.9		41.9		1987	b	422.5		14.9		41.2		—	—	3.28	b
SS : 2017	0.60		20.5		39.7		49.3		1184	d	211.6		16.5		39.3		2.3		2.80	b
SS : 2018	1.31		29.3		49.2		51.1		1275	d	168.1		19.8		27.1		1.7		7.97	a
DS : 2016	2.67		25.7		107.9		57.8		2200	a	960.4		22.2		93.9		—	—	3.34	b
DS : 2017	1.09		25.6		53.3		53.0		1242	d	774.4		19.9		90.6		4.8		1.67	b
DS : 2018	2.54		35.7		79.9		64.8		1783	c	921.6		24.4		82.5		5.2		3.13	b
p(soil)	< 0.0001	< 0.0001	0.0002	0.0013	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0012	
p(season)	< 0.0001	< 0.0001	< 0.0001	0.1153	< 0.0001	0.0224	0.0009	0.1658	0.8745	0.0001			0.0009		0.1658		0.8745		0.0001	
p(soil*season)	0.1316	0.6667	0.3592	0.2950	0.0006	0.1363	0.2048	0.9579	0.3978	0.0029			0.2048		0.9579		0.3978		0.0029	





**FIGURE 7.** Total anthocyanins (TA, Absorbance at 520 nm) (A) and Total polyphenols (TP, Absorbance at 280 nm) (B) in berry skins of shallow (SS) and deep soils (DS) vines during plants phenology (days after veraison, DAV) in seasons 2017 and 2018 under soil type, DAV and season interaction effect. Symbols are means  $\pm$  SEM ( $n = 12$ ) and different letters show statistic differences for the soil \* DAV \* season interaction (LSD Fisher;  $p < 0.05$ ) and are comparable between DAVs.



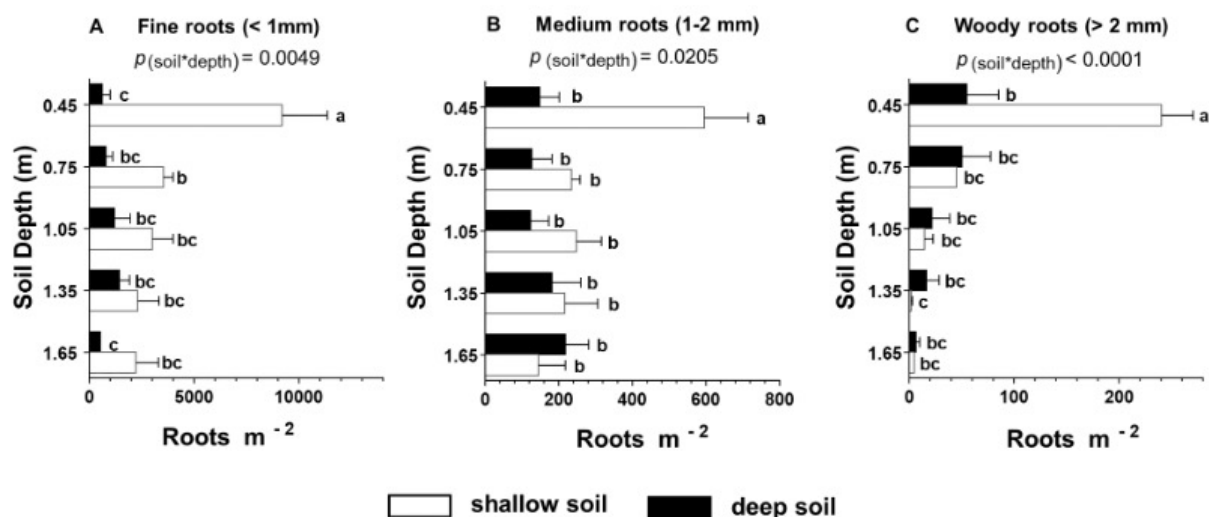
**FIGURE 8.** Total anthocyanins (TA, assessed as Absorbance at 520 nm) (A), Total polyphenols (TP, Absorbance at 280 nm) (B), and oxygen radical absorbance capacity (ORAC, mM of TE) (C) in berry skins of shallow (SS) and deep soils (DS) vines along with 2016, 2017 and 2018 seasons. Values are means  $\pm$  SEM ( $n = 12$ ) and different letters show statistic differences for the soil \* season interaction (LSD Fisher;  $p < 0.05$ ).

DS:  $1011.3 \pm 242$  roots  $m^{-2}$ ;  $p_{(soil)} = 0.0067$ ), but there was no difference in medium and woody roots between soils. The PCA showed that in SS soils low-fine root density sites were related to sand percentage, while high-root density spots were related to silt, clay, and organic matter content. Such soil characteristics did not affect the fine root density patterns in DS (Figure 10).

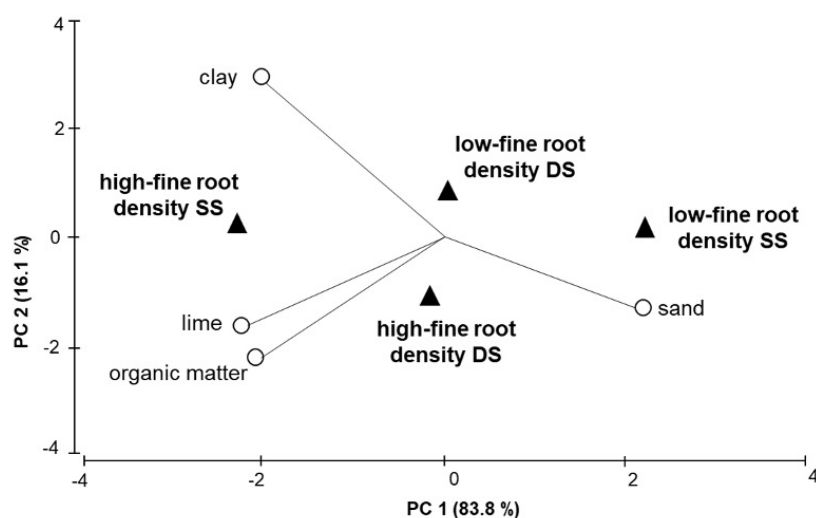
## DISCUSSION

Soil texture, structure, and depth play a major role in plant growth, anatomical adaptation development, berry quality

and root density and distribution (Morlat and Jacquet, 1993; van Leeuwen and Seguin, 2006; Reynolds *et al.*, 2013; Reynolds and Rezaei, 2014; van Leeuwen *et al.*, 2018; Priori *et al.*, 2019; Roi-Puscama, *et al.*, 2021; Gatti *et al.*, 2022). When considering plant-soil interactions, knowledge of the root system is particularly relevant in zoning the vineyard management (Brillante *et al.*, 2016; Fleishman *et al.*, 2019). Notwithstanding, it is challenging to determine effective rooting depth, especially on rocky soils (Smart *et al.*, 2006). Besides, coarse fragments frequency (skeleton) is the main feature discriminating SS and DS, as it impacts soil mass explorable by roots, nutrient and



**FIGURE 9.** Root density in shallow (SS) and deep (SD) soil at different depths. Values are means  $\pm$  SEM ( $n = 3$ ) and different letters show statistical differences for the soil \* depth interaction (LSD Fisher;  $p < 0.05$ ) within each root size category.



**FIGURE 10.** Biplot of principal component analysis of sand, silt, clay and organic matter variables, and high and low-fine root density sites in shallow (SS) and deep (DS) soils. Principal components 1 and 2 explain 99.9 % of the data variability.

water availability. Considering the predominant conditions in Argentina's vineyards of irrigated and own-rooted vines, the topic raises much interest. Recent publications refer to rooting capacity when calculating the AWC of soil (Priori *et al.*, 2020; Costantini, 2021), and other techniques have been used to detect functional rooting depth which can reach deeper depths, being highly variable during the different phenological stages of the grapevines (Karakis *et al.*, 2017; Savi *et al.*, 2019). In the present work, the environmental differences between shallow and deep soils, such as mean air temperature, relative humidity and solar perception at bunch level, might be attributable to disparities in canopy architecture. According to previous results (Roig-Puscama *et al.*, 2021), Malbec plants acclimate to the edaphic environment by developing a larger hydraulic conduction capacity in SS to overcome conditions of low

soil water retention capacity. Root distribution in both soils reached a depth of at least 1.65 m (no deeper exploration was performed). The stony nature of the SS and the rocky layers underneath did not seem to impede vertical growth at depth. This has also been reported in dry-farmed gravelly soils, deep coarse sands, and even limestone soils where roots can grow as deep as  $> 6$  m (Smart and Coombe, 1983; Savi *et al.*, 2019). The fact that the first 0.45 m of SS held most of the vine root system is likely to be associated with better availability of water and nutrients (Swanepoel and Southey, 1989; Smart *et al.*, 2006).

This is a reasonable assumption since the SS contained 77 % of stones below this depth. However, the presence of roots at 1.65 m depth could be a strategy to ensure access to water resources, as proposed by van Leeuwen *et al.* (2004). In contrast, DS plants had a more uniform distribution in

terms of total root size category throughout the soil profile and less total root quantity. Bordoní *et al.* (2016) also found a lower grapevine root density when studying low permeable soils of Croatina, Uva Rara and Barbera in several soil profiles ranging from moderately well- to well-drained in Italy. Considering all depths, vines developed finer roots in SS than in DS, possibly as a consequence of abscisic acid (ABA) synthesis due to limited water availability (Lovisolo *et al.*, 2016). In addition, the high-fine root density patches in SS were related to higher contents of soil organic matter, clay, and silt, while low-fine root density sites were related to higher sand. These physico-chemical conditions probably represented a “hot spot” of better water and nutrient availability, promoting the development of fine roots, while soil regions of higher percentage of sand seemed to be unsuitable for the establishment of fine roots. Patches of high and low density of fine root in DS were not related to any physico-chemical property studied. Possibly, when soil resources are not a limiting factor, this “hot spot” distribution of fine roots is not as common.

Root morphology and distribution were related to variations in canopy size. It is possible to envisage that the numerous root systems in SS plants is produced at the expense of a reduced canopy, affecting the bunch environment.

Differences in bunch environment were also more conspicuous from January (post fruit set stage) onward, once the canopy’s final size was established, suggesting an association with vegetative expression. In fact, the reduced foliage in SS vines allowed the greatest interception of UV-B and PAR radiation at bunch level, while the denser and taller canopies of DS plants may have blocked part of these spectra. Thus, higher radiation may have led to a warmer and lower relative humidity in the air surrounding the bunches in SS plants, from veraison to harvest. Low relative humidity values could also be partly explained by the improvement in air removal due to low SS vegetative growth. Extreme thermal events, such as temperatures  $\geq 35^{\circ}\text{C}$ , have been reported in vineyards from different locations around the world. Studies on Cabernet Sauvignon from California (USA), Shiraz from Victoria (Australia) and even the Champagne region (France) have addressed this topic to consider mitigation strategies for the future (Briche *et al.*, 2011; Zhang *et al.*, 2015; Martínez-Lüscher *et al.*, 2020). In our study, sporadic events with temperatures  $\geq 35^{\circ}\text{C}$  at bunch level were more frequent in SS plants in 2017 only (data not shown). This possibly occurred due to less water availability for transpiration as indicated by the  $\Psi_s$  dynamics. Regarding soil thermal status from January to March in 2018, SS was warmer than DS. The reduced volume of SS soil and its coarse nature affecting the soil water content could have contributed to a greater heat absorbance and transmission (Campbell and Norman, 2012) compared to DS. Furthermore, this could be partially explained by the higher solar radiation intensity received in contrast to the canopy blocking effect in DS. Differences in phenology and fruit ripeness are related to soil variability, as demonstrated by Sams *et al.* (2022), who used remotely sensed imagery in Cabernet Sauvignon vineyards from

California over three vintages. In a study on Cabernet Sauvignon and Chardonnay from two vineyards in Chile, Verdugo-Vásquez *et al.* (2022) found that soil apparent electrical conductivity and topography were the main factors to affect phenology and soluble solid accumulation. In this study, maturity occurred from 9 to 14 days earlier in SS than in DS vines. In the coldest season (2016), berries were harvested at a lower maturity level because of snow risks, but the same level of maturity was achieved in both treatments. This anticipation could have been caused by a warmer root zone temperature registered from January onwards, accompanied by a limited soil-water availability, as pointed out by van Leeuwen *et al.* (2018). Another three-year study in dry-farmed conditions also found precociousness in ripening on shallow soil when comparing three soil types with increasing depths (Bodin and Morlat, 2006). Conversely, de Andrés-de Prado *et al.* (2007) did not find any differences in ripening in two contrasting soils located 500 m away from each other in a two-year trial. In our experiment, the earlier occurrence of budbreak in 2017 could have been a response to warmer temperatures in the early spring (September) compared to 2018. The lack of differences in budbreak and full bloom appearance between both soil types can be explained by the absence of significant differences in MMAT between September and December. However, the synchronic occurrence of veraison in both soils and seasons remains to be explored.

The stem water potential values measured in 2017 suggest that SS plants could have struggled with low water intake given the physical constraints of soils. In that season, differences between SS and DS in terms of  $\Psi_s$  allied with differences in terms of MMRH, MMAT and MMST from pre-veraison to harvest (January onwards). Coipel *et al.* (2006) found average lower predawn leaf water potential values in shallow soils compared to deep soils in French vineyards. This hydric situation suffered by SS plants, in combination with other environmental stresses such as high UV-B radiation, blocks the normal development and reproductive processes, like those reviewed in Kakani *et al.* (2003) for several species. For instance, the low RCC values in SS plants might have been caused by photooxidation and consequent chlorophyll degradation, a response which was found to increase in Sultanina when submitted to 15 % more UV-B and 50 % less irrigation than the control treatment (Doupis *et al.*, 2020). This may have also reflected a response to temperatures, high levels of UV-B, saturating PAR radiation and water stress interactions. Light intensity at bunch level could have exceeded the light compensation point given the low photosynthetic rates caused by high temperatures and water scarcity. The limited photosynthesis rate caused by stomata closure due to water stress may also have led to an energy excess that provoked oxidative stress. This might have similarly affected photosystem integrity ( $F_v/F_m < 0.725$ ; Critchley, 1998), which was detected in SS plants in 2017. Our results partially agree with previous studies. Zulini *et al.* (2005) found that water stress did not affect  $F_v/F_m$ , but it diminished RCC in *V. vinifera* cv. Riesling, concluding that photosynthetic efficiency is only hampered

by prolonged periods of drought stress. However, in high-altitude vineyards, the conjunction of common abiotic stresses, such as high solar UV-B and PAR radiation and stony and easily dryable soils, along with extreme and sporadic events of high temperatures on low vigour vines, could have accelerated the alteration of the photosystem.

Our results showed that vegetative and yield-related variables were affected by soil type. The overall better water status of the DS plants, as well as other processes, could have allowed adequate cell elongation, stomata openness and photosynthesis rate. These processes could explain the longer shoots and greater LA, as well as the heavier prune weight and part of the yield components in DS-grown grapevines. The effects on bunch FW were also most likely related to water stress, as was previously observed by Matthews and Anderson (1989). The greater number of bunches per plant of DS vines responded to the presence of more suckers, compared to SS, since pruning criteria was the same for both treatments. On the other hand, according to Guilpart *et al.* (2014), flowering is the critical water sensitivity period in the number of berries per bunch formation process. Since there were no records of  $\Psi_s$  during this phenological stage, is it not possible to relate water stress with the observed reduction of the number of berries per bunch in SS plants. However, this possibility cannot be ruled out given the frequent moderate and severe water stress experienced by these plants during the recorded months. Since the number of berries and the number of bunches per plant are considered the main drivers of yield (Guilpart *et al.*, 2014), they could explain the yield depletion in SS. Plants grown in SS showed a reproductive/vegetative balance favouring fruit yield compared to DS plants represented by the Ravaz index, as previously reported by van Leeuwen *et al.* (2004). As expected, the different seasons also played a major role in the changeability of vegetative and yield-related variables, although season and soil type interaction were only observed in the berry FW and Ravaz index. This suggests a strong influence of soil type on the studied vegetative and yield-related variables.

In 2018, even when environmental conditions measured at bunch level and water stress were not as limiting as in 2017, SS vines showed a reduction in vegetative growth and an increase in berry phenolics and antioxidant capacity. This scenario was also observed in 2016, a climatological milder season, suggesting that plant vigour and metabolic activity in high-altitude viticulture can be affected by other environmental factors, such as UV-B radiation (Alonso *et al.*, 2016), when water and temperature are not limiting. At the 2017 harvest, DS vines presented more TP with no differences in TA compared to SS vines. It is possible that the combination of water stress, sporadic events with temperatures  $\geq 35^\circ\text{C}$  and augmented UV-B radiation at bunch level, had favoured the degradation of phenolics in berry skins of SS-grown vines. De Rosas *et al.* (2017) proved in a field experiment on Malbec and Bonarda that a 2–3  $^\circ\text{C}$  temperature increase not only caused a general loss of TA berry content but also an increase in the proportion of acylated anthocyanins. A previous study in the same vineyard and variety demonstrated that berries

exposed to ambient UV-B induced a higher accumulation of TP and TA compared to those under minor UV-B intensity (Berli *et al.*, 2015). Thus, part of the present results could be explained by a UV-B stress response, since the UV-B irradiation in DS bunches is reduced compared to that in SS. Irrespective of the season, ORAC was consistently higher in SS berries, although in 2017 it diminished, possibly due to a combination of UV-B, water stress and high-temperature. A former experiment, in the same vineyard, also showed an increase in ORAC caused by the effect of UV-B, but only on well-watered plants, since the combination of water restriction and UV-B decreased these phenolic compounds (Alonso *et al.*, 2016). Current winemaking experiments are being carried out to find relationships between soil plots, wine chemistry and organoleptic features.

The ongoing changing environment pushes viticulturists and oenologists to explore new lands and discover different terroirs for premium wines. This study provides information on Malbec behaviour in high-altitude vineyards characterised by soils with rocky bases of different depths and high levels of UV-B radiation. Results suggested that in shallow high-altitude soils, the microclimate in Malbec plants may provoke a considerable abiotic stress level. Under this situation, berry quality is maintained or even increased as a trade-off between vegetative and reproductive expression. However, special attention to irrigation is necessary to overcome potential constraints in plant growth and grape quality.

An exhaustive soil exploration at the foot of the Andes Mountain range is required to individualise homogeneous conditions for plot delimitations to apply irrigation programmes that fulfill specific crop needs. The present work provides information as a starting point for management strategies in high-altitude vineyards that will strengthen the viticultural sector.

## CONCLUSION

For the first time, the effects of shallow versus deep soil on root growth within a single high-altitude Malbec vineyard were investigated on two vintages and in three growing seasons. In shallow soil, root systems were not limited by physical constraints and the total amount of roots was higher than in deep soil. In addition, fine root density patterns were concentrated within patches containing a higher proportion of clay, silt, and organic matter, while fine roots from deep soil were not related to such characteristics. Grapevines in shallow soil were oriented to root production at the expense of vegetative growth; the morphophysiological stress responses, such as reduced vegetative growth and fruit yield, were accompanied by high contents of polyphenols in berry skins. However, vines subjected to moderate to severe water stress could not cope with extreme temperatures and high UV-B and PAR radiations, showing physiological damage. The results suggest that high-altitude features, such as high UV-B and vertical soil heterogeneity, in combination with events of high temperatures and water restrictions, need to be



carefully considered when developing vineyard management strategies.

**Author's contribution:** D. S. Mezzatesta, F. J. Berli, and P. Piccoli conducted the experiment and carried out the physiological and biochemical analyses. D. S. Mezzatesta, F. J. Berli and C. Arancibia conducted statistical analyses. F. Buscema and P. Piccoli collaborated in finding funding and deciding strategies. D. S. Mezzatesta, F. J. Berli, P. Piccoli and C. Arancibia wrote the body of the paper. All authors reviewed, edited and approved the final version of the manuscript.

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