Drought stress and soil management practices in grapevines in Cyprus under the threat of climate change

Antonios Chrysargyris, Panayiota Xylia, Vassilis Litskas, Athanasia Mandoulaki, Demetris Antoniou, Timos Boyias, Menelaos Stavrinides and Nikos Tzortzakis

ABSTRACT

The Middle East, the cradle of viticulture and wine production, is gradually but steadily becoming hotter and drier because of climate change (CC). In the current study, we evaluated the effect of tillage and irrigation on yield and quality characteristics of the heat-resistant, indigenous red-grape variety Maratheftiko for one year. Yield increased (two-fold) in vines with irrigation and tillage compared to tillage with no irrigation. The absence of tillage buffered the negative effect of the lack of irrigation on yield. At the veraison stage, leaf stomatal conductance decreased in non-irrigated vines, independently of the application of tillage or not. At veraison, tillage increased (up to 27.5%) phenolics when compared to no tillage in non-irrigated vines. Vines accumulated more N, P, and K and less Mg during the flowering stage compared to veraison. At veraison, irrigation decreased K content in vines subjected to tillage and decreased Mg content in vines subjected to no tillage. Total soluble solids and anthocyanins of berries increased with the absence of irrigation and tillage. Total phenolics increased with tillage in both irrigated and non-irrigated plants. Our results indicate that no tillage systems may be viable as an adaptation strategy in the context of CC.

Key words | cultivation practices, grapes, irrigation, phenolics, quality, tillage

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INTRODUCTION

The Middle East, the cradle of viticulture and wine production, is gradually but steadily becoming hotter because of climate change (CC) (Evans 2009; Lelieveld *et al.* 2016). Heat extremes are expected to increase with the maximum temperature during the hottest days reaching 50 °C by the end of the century under the business as usual scenario (Radiative Concentration Pathway, RCP, 8.5), which means radiative forcing in the Earth's atmosphere $+8.5\ W/m^2$ in the year 2100 relative to pre-industrial values (Lelieveld *et al.* 2016). In most countries of the Middle East, heating is accompanied by drying and an increase in reference evapotranspiration (Terink *et al.* 2013). Although many indigenous varieties of grapes originating from the region have been hand-selected for millennia for their resistance to heat and drought, the changing climate will push plants to their limits, impacting the quality and quantity of yields (Fraga *et al.* 2016). Among the most important effects of CC on vine are advanced harvest times, increased grape sugar concentrations leading to high wine alcohol levels and lower acidities (de Orduna 2010). Vine metabolism may be inhibited at high temperatures leading to reduced metabolite accumulations, which may affect wine aroma and color and increase the risk of spoilage and organoleptic degradation.

Viable adaptation strategies for coping with water stress in Middle Eastern vineyards need to focus on conserving the declining amounts of water available to vines through rainfall or irrigation (Terink *et al.* 2013). Excluding irrigation,

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soil management is the most important practice that may influence water availability to grapevines (Steenwerth et al. 2016). In the last 20 years, the use of no tillage systems in vineyards has gained increasing attention because of benefits in reducing soil erosion (Novara et al. 2011), lowering dust generation (Baker et al. 2005), managing global warming potential (Wolff et al. 2018), and providing biological control services (Daane et al. 2018).

Tillage affects soil's physical and chemical properties such as micro- and macro-pore distribution that determine air, water, and heat transfer processes in the root zone. Moreover, tillage affects the concentration and decomposition rate of organic matter which determines the microbiological activity in the soil, which is crucial for vine nutrition (Pérez-Bermúdez et al. 2015). Tillage as well as irrigation is also very important for nutrient uptake (e.g., N, P, K, Mg) by the vines (Keller 2005). Tillage at an increased depth (e.g., 25 cm) in early spring could reduce the available water for the vine by exposing deeper and more wet soil zones to more sun radiation which increases evaporation losses. On the other hand, surface tillage (0–10 cm) in hot months could disrupt the continuity of micropores resulting in reduced movement of soil water to the surface, due to the effect on capillary fringe (Beis & Patakas 2015).

Furthermore, tillage affects grape plant physiology, growth, and production, as well as the qualitative characteristics of berries (Trigo-Córdoba et al. 2015). Polyphenol composition and concentration in grapes and wines is influenced by different factors including soil conditions (Deis & Cavagnaro 2013). The accumulation of anthocyanins, the red pigments in the skin of red varieties, begins at veraison and is one of the most commonly recognized features of berry ripening. The pattern of anthocyanin accumulation in grapes tends to follow that observed for sugar accumulation, but can differ drastically depending on variety, site, and cultural management practice (de Orduna 2010). Tannins are naturally occurring compounds that exist inside grape skins, seeds, and stems. Tannins are complex polymeric compounds that are responsible, at least in part, for many of the sensory attributes of wines, particularly red wines. They also work as antioxidants and protect the wine from deterioration in quality (Deis & Cavagnaro 2013). The composition of these compounds in grapes may change in response to tillage practices (Trigo-Córdoba et al. 2015).

Cyprus has the oldest wine tradition in the Mediterranean area, with more than 5,500 years of wine production. Vineyards cover 7,000 ha of High Nature Value Farmland on the island. Climate change projections for Cyprus show an increase of heat extremes and a decrease in precipitation, as is the case for many other countries in the region (Terink et al. 2013; Lelieveld et al. 2016). There are more than ten indigenous grape varieties on the island, with many of them very well adapted to drought. Indigenous varieties require less input (e.g., water, fertilizers) in comparison to introduced varieties (Litskas et al. 2017), and offer promising prospects for adaptation to climate change. The changing climate, together with restrictions in the amount of irrigation water and a shift in consumer preference (87.2% as reviewed by Vrontis & Papasolomou 2007) towards local products, led to the rediscovery of rare indigenous varieties, including the red variety Maratheftiko. Its cultivation is slowly on the increase again (Ministry of Agriculture, Rural Development and Environment, personal communication) as it offers a distinctive character to local wines.

The current main practice in Cyprus is the tillage of vineyards two to three times per year to remove the ground weed cover because of the perceived competition for water between the growing weed vegetation/flora and vines. In recent years, some wineries have begun to leave the weed vegetation/flora growing naturally between rows, especially in vineyards with Maratheftiko, to reduce the vegetative growth of plants. However, nothing is known about the impact of irrigation and cultivation practices on Maratheftiko plant growth and yield characteristics. The current study aimed at evaluating the effects of tillage (tillage vs no tillage) and irrigation (irrigation vs no irrigation) on plant growth, yield, and berry quality characteristics of the Maratheftiko variety. In this study, we used as a model the island of Cyprus, in the eastern part of the Mediterranean basin.

MATERIALS AND METHODS

Plant material and treatments

The 11-year-old own-rooted grapevines (*Vitis vinifera* L. cv. Maratheftiko) were used under field conditions, during the year of 2017 at the Malia winery commercial vineyards, Limassol, Cyprus (34°49′N, 32°47′E, 645 m). Maratheftiko does not have hermaphrodite flowers like many cultivated grape varieties and requires co-planting with other varieties (i.e., Spourtiko) to achieve fertilization and fruit development. The vineyard occupied approximately 0.6 ha, and the soil, according to FAO classification, is a clay-loam, with 2.21% organic matter; available $CaCO_3$ 67.3%; pH 7.46; EC 0.27 mS/cm. The climate of the region is dry with less than 30 mm of summer rainfall (June to August) while the average midday temperature and air humidity during the summer months of on-vine ripening were about 30.0 °C and 30%, respectively. Microclimatic parameters were continuously recorded by a meteorological station, which was located in the experimental vineyard. The recent five-year meteorological data are presented in the Supplementary material (Figure S1, available with the online version of this paper).

All plants were trained in a traditional bilateral 'royat' system. Vine spacing was 1.50 m in north–south-orientated rows with 2.4 m between the rows at a plant density of 2,700 plants/ha. The main wire was 0.70 m above the soil surface and the shoots were maintained on a vertical plane by three wires, the highest of which was located 1.60 m above the soil surface. All vines were uniformly pruned and were irrigated with a drip irrigation system. The plants are frequently tested by local authorities for virus infection as Cyprus is free of *Dactylosphaera vitifoliae* (Shimer). Common phytochemical practices were applied to minimize insect or disease pathogen infection.

From the beginning of spring (March 2017), the experimental plot was divided into four treatments: tillage and no tillage crossed with irrigation and no irrigation. Therefore, the resulting treatments were (i) no irrigation and no tillage (no irrig/no tillage), (ii) with irrigation and no tillage (with irrig/no tillage), (iii) no irrigation and tillage (no irrig/tillage), and (iv) with irrigation and tillage (with irrig/tillage). In the no tillage treatments, plants growing naturally on the vineyard floor were allowed to develop. Each treatment consisted of four plots (five vines each; 20 vines per treatment). Guard rows between treatments was the Spourtiko cultivar, necessary for flower fertilization. The amount of irrigation applied was programmed according to the soil volumetric water content (VWC) ranging from 20% to 30% of the irrigation/tillage treatment. The targeted VWC was 25% and was optimized by preliminary trials of the same soil in pots, irrigated up to maximum water holding capacity. Soil VWC was measured by field-scout TDR300 with 20 cm rods (Spectrum Technologies Inc., Aurora, IL, USA). Irrigation water was supplied approximately every 10–15 days. Soil water content measurements took place from May 2017 to September 2017 at 15-day intervals, to capture the variability of soil moisture content through the season (Figure 1).

Measurements

Cover crop biomass

Above ground cover crop biomass (natural vegetation) was sampled in May 2017. All cover crop biomass present within a 1 meter square quadrat was removed with four samples per treatment. Squares were selected randomly. The percentage of area covered by natural vegetation per square meter, plant fresh and dry weight (in g), total nitrogen (g kg^{-1}), and organic matter (%) were determined.

Plant growth, production, and physiological parameters

Physiological and photosynthetic parameters were measured at flowering (May), veraison (July), and harvesting (September) with four replicates/treatment. Stomatal conductance was measured on the 4th to 5th leaf from the top of the plant (three measurements per leaf). All leaves were fully

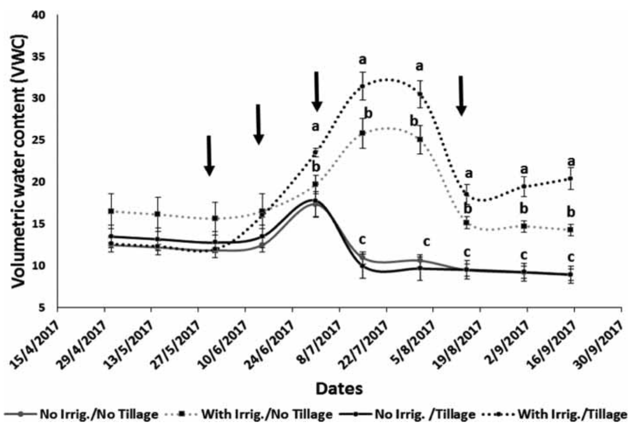

Figure 1 | Soil volumetric water content in the four treatments. Arrows show the dates irrigation was applied. Significant differences ($P < 0.05$) among treatments are indicated by different letters according to Duncan's multiple range tests. Error bars show SE ($n = 4$).

mature and sun-exposed in different individual plants per treatment. Stomatal conductance measurements were carried out using a ΔT-Porometer AP4 (Delta-T Devices-Cambridge, UK) according to the manufacturer's instructions.

Leaf tissue (four replications/treatment; each replication consisted of a pool sample of two plants; 0.1 g) was incubated in a heat bath at 65 °C for 30 min, in the dark, with 10 mL dimethyl sulfoxide (DMSO) for chlorophyll extraction. Photosynthetic pigments, i.e., chlorophyll a (Chl a), chlorophyll b (Chl b), and total chlorophyll (t-Chl) contents were calculated as described by Chrysargyris *et al.* (2017a). Maximum F_v/F_m photochemical quantum yields of PSII were measured with an OptiSci OS-30p Chlorophyll Fluorometer (Opti-Sciences). Leaves were incubated in the dark for 20 min prior to F_v/F_m measurements.

During harvest (September), the number of clusters per plant, the grape fresh weight, and the yield (kg $plant^{-1}$) were measured in four replicate samples (each replicate had three plants) per treatment.

Polyphenol content and antioxidant activity in leaves

Polyphenols and antioxidant activity were measured at flowering (May), veraison (July), and harvesting (September). The total phenolic content was determined with the Folin–Ciocalteu method at 755 nm according to Klados & Tzortzakis (2014) and results were expressed as equivalents of gallic acid (Scharlau, Spain) per g of fresh weight (mg of GAE g^{-1} Fwt). The antioxidant capacity using the ferric reducing antioxidant power (FRAP) and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) methods as well as total flavonoids content were performed as previously described (Chrysargyris *et al.* 2016, 2017b). The results for antioxidant activities were expressed as equivalents of trolox per g of fresh weight (mg trolox g^{-1} Fwt) and for the content of total flavonoids as rutin equivalents (mg rutin g^{-1} Fwt).

Plant nutrient content analysis

The mineral content in leaves was measured at flowering (May) and veraison (July). Leaf stem tissue (four replicates/treatment) was dried at 65 °C for 4 d, weighed, and grounded in a Wiley mill to pass through 40 mesh screens (as described in Marinou *et al.* 2013). The determination of K was made using a flame photometer (Lasany Model 1832, Lasany International, India), of P in a spectrophotometer (Multiskan GO, Thermo Fischer Scientific, USA), of Mg by an atomic absorption spectrophotometer (PG Instruments AA500FG, Leicestershire, UK), and of N by the Kjeldahl method (BUCHI, Digest automat K-439 and Distillation Kjelflex K-360, Switzerland). Data were expressed in g kg^{-1} of dry weight.

Qualitative attributes

Sampling of berries

Berries were collected at harvest in nylon bags (100 berries from each of the four plots/treatment), when sugar concentration reached c. 24°Brix. In the field, samples were kept in ice to prevent dehydration. In the laboratory, berries from all treatments were weighted and then either used for measurements or were frozen and conserved at −20 °C until analysis.

Soluble solids content, titratable acidity, and pH

Total soluble solids (TSS), titratable acidity (TA), and pH were determined according to the methods described by the International Organization of Vine and Wine (OIV 2012). For the determination of TSS, a portable digital refractometer (Master Baume 2594, Atago, Japan) was used. The determination of TA was conducted by potentiometric titration with 0.1 mol L^{-1} NaOH up to pH 8.1, using 5 mL juice diluted in distilled water until a final volume of 25 mL. The measurements were carried out using a DL22 Mettler Toledo titrator (Mettler-Toledo, Inc., Columbus, Ohio, USA). The pH values were measured with a pH-meter (HI 2222, Hanna instruments, Inc., Woonsocket, Rhode Island, USA). All measurements were carried out in triplicate.

Ascorbic acid content

Ascorbic acid content was determined by the 2,6-dichloroindophenol titration method according to Deng *et al.*

(2005) and results were expressed as mg of ascorbic acid per 100 mL of grape juice (mg of AA 100 mL^{-1} grape juice).

Polyphenols, anthocyanins, and tannins in berries

Polyphenols and anthocyanins were extracted by a modified method described by Du *et al.* (2012). Fresh grapes were homogenized with 80% acetone and extraction was assisted with an ice sonication water bath for 10 min. Samples were centrifuged at 4,000 *g* at 4 °C for 15 min and supernatants were stored at −20 °C until use for analysis of total phenolics and anthocyanins. Total phenolic content was measured as described above and results were expressed as equivalents of gallic acid per g of fresh weight (mg of GAE g^{-1} Fwt). The total anthocyanin content of the grape extract was determined with the pH differential method at 520 and 700 nm according to Du *et al.* (2012) and results were expressed as equivalents of cyaniding 3-glucoside per 100 g of fresh weight (mg of cyn-3-glu g^{-1} Fwt). Condensed tannins were determined using the Bate-Smith assay as described by Bate-Smith (1981) at 550 nm and results were expressed as g of condensed tannins per liter of grape juice (mg of condensed tannins per 100 mL grape juice).

Statistical methods

Statistical analysis was performed using IBM SPSS version 22 comparing using one-way analysis of variance (ANOVA) and Duncan's multiple range tests for comparisons of treatment means at $P < 0.05$. Measurements were done in four biological replications/treatment (each replication consisted of a poll of three individual measures/samples).

RESULTS

Biomass production from cover crop

The weed soil cover was 68.7% and the main species were from Poaceae (*Aegilops* sp., *Bromus* sp.), Asteraceae (*Sonchus* sp.), Malvaceae (*Malva* sp.), Fabaceae (*Medicago* sp.), and Brassicaceae (*Hirschfeldia incana*, *Capsella bursa-pastoris*, *Hirschfeldia incana*). Total biomass yield was 806.9 g m^{-2} for cover crop with a 40% dry matter content. Organic matter content was almost 93% and the content of total nitrogen was 11.8 g kg^{-1}.

Plant growth and physiology

Grapevine yield was increased (almost doubled) with irrigation for tillage cultural practice, and this increase was related mainly to the increased fresh weight of each cluster rather than the number of clusters produced (Table 1). In case of vines under no tillage, irrigation did not have a significant effect either on crop yield or on number and weight of clusters. Irrigation significantly increased yield only for vines in the tillage treatment.

No differences were found in the content of chlorophylls and leaf stomatal conductance at the flowering stage (May) of vines in the four treatments (Figure 2). At veraison, leaf stomatal conductance decreased (up to 73%) in non-irrigated vines, independently of the application of tillage or not. Irrigated vines subjected to tillage revealed a 41% decrease in leaf stomatal conductance compared to no tillage treatment. No differences were found in chlorophylls' content (Figure 2) and in leaf fluorescence (data not presented). At harvest, the content of Chl a and

Table 1 | Effects of irrigation and tillage on the number of clusters per plant, cluster weight (g $plant^{-1}$) and yield (kg $plant^{-1}$)

		No. of clusters	Cluster weight	Yield
No irrigation	No tillage	25.75 ± 2.59 a	137.17 ± 3.37 ab	3.54 ± 0.43 ab
	Tillage	23.25 ± 2.92 a	117.61 ± 14.44 b	2.48 ± 0.58 b
Irrigation	No tillage	28.41 ± 3.45 a	135.35 ± 15.54 ab	3.77 ± 0.52 ab
	Tillage	26.74 ± 3.25 a	166.52 ± 21.01 a	4.54 ± 0.96 a

Values ($n = 4$) in columns followed by the same letter are not significantly different at $P \leq 0.05$ according to Duncan's multiple range tests.

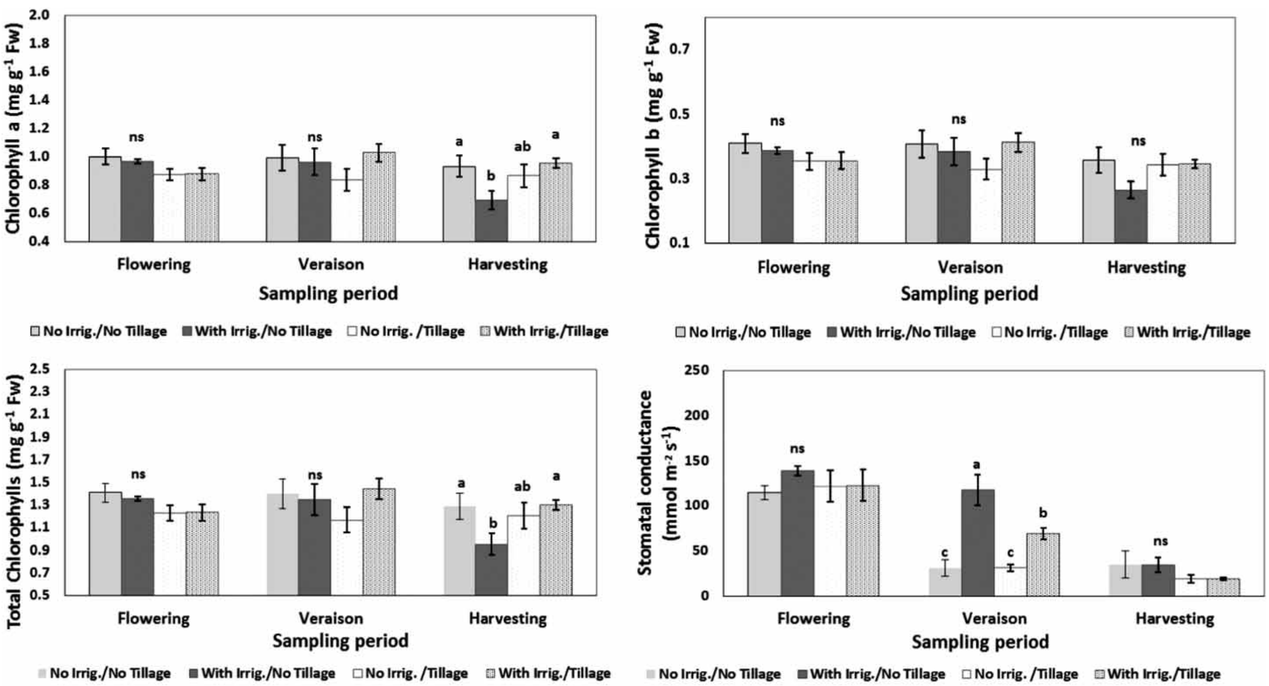

Figure 2 | Impact of irrigation and tillage on chlorophyll a (Chl a; mg g^{-1} fw), chlorophyll b (Chl b; mg g^{-1} fw), total chlorophylls (total Chl; mg g^{-1} fw), and leaf stomatal conductance (mmol m^{-2} s^{-1}). Sampling dates were during flowering (1st sampling), veraison (2nd sampling), and harvesting (3rd sampling). Significant differences ($P < 0.05$) among treatments are indicated by different letters according to Duncan's multiple range tests. Error bars show SE ($n = 4$).

therefore the total Chls content was significantly lower in irrigated vines under no tillage vs the no irrigation/no tillage and irrigation/tillage treatments.

Polyphenols and antioxidant activity

At veraison, irrigation with tillage led to increased antioxidant capacity obtained by FRAP method, compared to no irrigation with no tillage. At harvest, the application of irrigation with no tillage decreased antioxidant activity, measured by FRAP and ABTS methods (Figure 3), compared to the no irrigation/no tillage treatment. In general, we observed an increase in antioxidant capacity during plant development, as the plants are passing from flowering to veraison and then to the harvesting stage. A higher content of phenols was observed in the irrigation/tillage treatment compared to no irrigation/no tillage, at the veraison stage. No differences were found during flowering for the content of total phenolics as well as antioxidant status, as measured by FRAP and ABTS methods.

Mineral content in commercial Mratheftiko grapevines

Vines accumulated more N, P, and K and less Mg during flowering compared to veraison (Figure 4). At flowering, no irrigation/tillage and with irrigation/tillage application decreased (up to 27%) the K content in plants compared to no irrigation/no tillage and with irrigation/no tillage treatments, while irrigation had no profound effects. The content of N, P, and Mg was similar among treatments at flowering. At veraison, irrigation decreased accumulated K in vines subjected to tillage and decreased Mg content in vines subjected to no tillage. Tillage application decreased (up to 27%) P content compared to no tillage, independently of the irrigation practice applied.

Quality attributes

The effects of the four treatments on quality attributes of grape juice are shown in Table 2. TSS increased in the no irrigation/no tillage treatment compared to irrigation/tillage and irrigation/no tillage. The content of total phenols increased with tillage application compared with

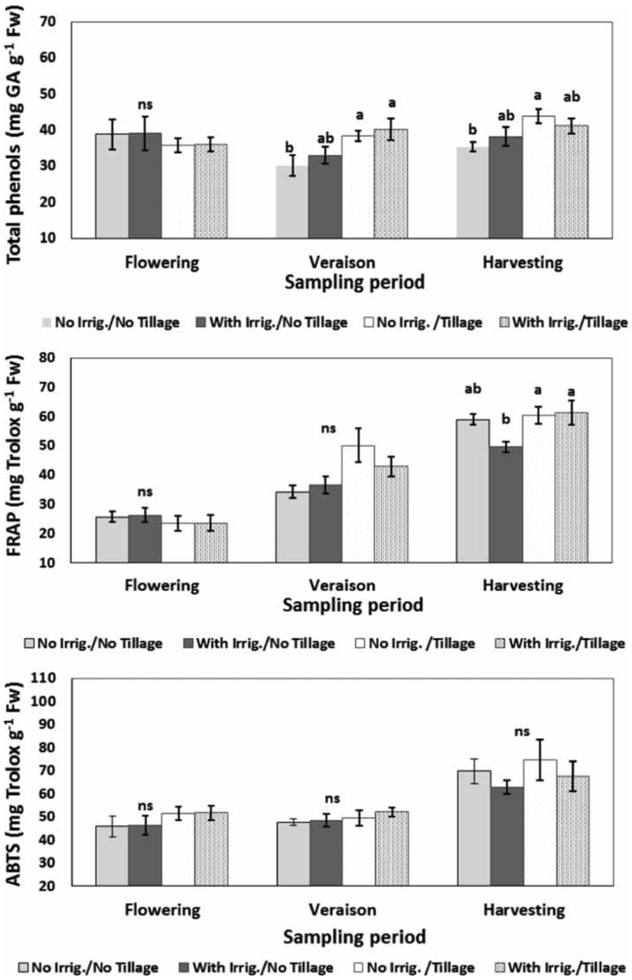

Figure 3 | Impact of irrigation and/or cultivation practices (cover crop – tillage) on the content of total phenols and antioxidant activity (FRAP, ABTS). Sampling dates were during flowering (1st sampling), veraison (2nd sampling), and harvesting (3rd sampling). Significant differences ($P < 0.05$) among treatments are indicated by different letters according to Duncan's multiple range tests. Error bars show SE ($n = 4$).

the no tillage practice in both irrigated and non-irrigated plants. The higher content of anthocyanins was found in the no irrigation/no tillage treatment, being significantly higher than the no irrigation/tillage and irrigated vines irrespective of tillage application. The content of tannins was significantly increased in irrigated vines under no tillage compared to all other treatments. In irrigated vines, cover crop practice resulted in lower grape juice pH compared to the tillage application. No differences were found in titratable acidity and the content of ascorbic acid among the treatments.

DISCUSSION

The natural vegetation in the no tillage treatment consisted of species that are common in Cyprus and the Mediterranean area (*Aegilops* sp., *Bromus* sp., *Sonchus* sp., *Malva* sp., *Medicago* sp., *Hirschfeldia incana*, *Capsella bursa-pastoris*, *Hirschfeldia incana*). The species are typically found in vineyards and olive groves and form a multispecies cover crop (Gómez *et al.* 2011). Biomass yield in our study is similar to that measured in Mediterranean olive groves and vineyards (Soriano *et al.* 2016).

Grapevine yield almost doubled with irrigation in the tillage treatment and this was mainly due to the increase in the fresh weight of each cluster, rather than the number of clusters produced. The effects of drought (no irrigation), a situation that it is expected for Cyprus under climate change, indicate a potential loss of more than 45% in yield under tillage (Table 1). The observed yield in the irrigation and tillage treatment was within the average yield observed in three years (2014–2016) in the same vineyard, and this ranged from 4.01 to 5.18 kg/plant (unpublished data). In the case of severe water shortage, no tillage and the presence of natural vegetation as cover crop could be a valuable option for reducing the yield loss for Maratheftiko. The presence of cover crop, when irrigation was applied, increased water infiltration compared to tillage and the irrigation water could not reach the roots of the Maratheftiko vines. In addition, the cover crop utilized a part of the irrigation water resulting in reduced water availability for the vines. In agreement with our observations, Barroso *et al.* (2016) worked with the Portuguese native variety 'Trincadeira' and concluded that the higher water availability due to irrigation and soil tillage during berry development resulted in an increase in berry flesh weight. Berry size and crop yield are widely recognized as important factors that contribute to wine quality.

There were no differences in stomatal conductance and chlorophyll content (chlorophyll a, b, and total) at flowering. Rain during Maratheftiko flowering (April–May) is usually adequate in the viticultural areas of Cyprus to support the evapotranspiration requirements, which are much lower than those observed later during the hot, dry

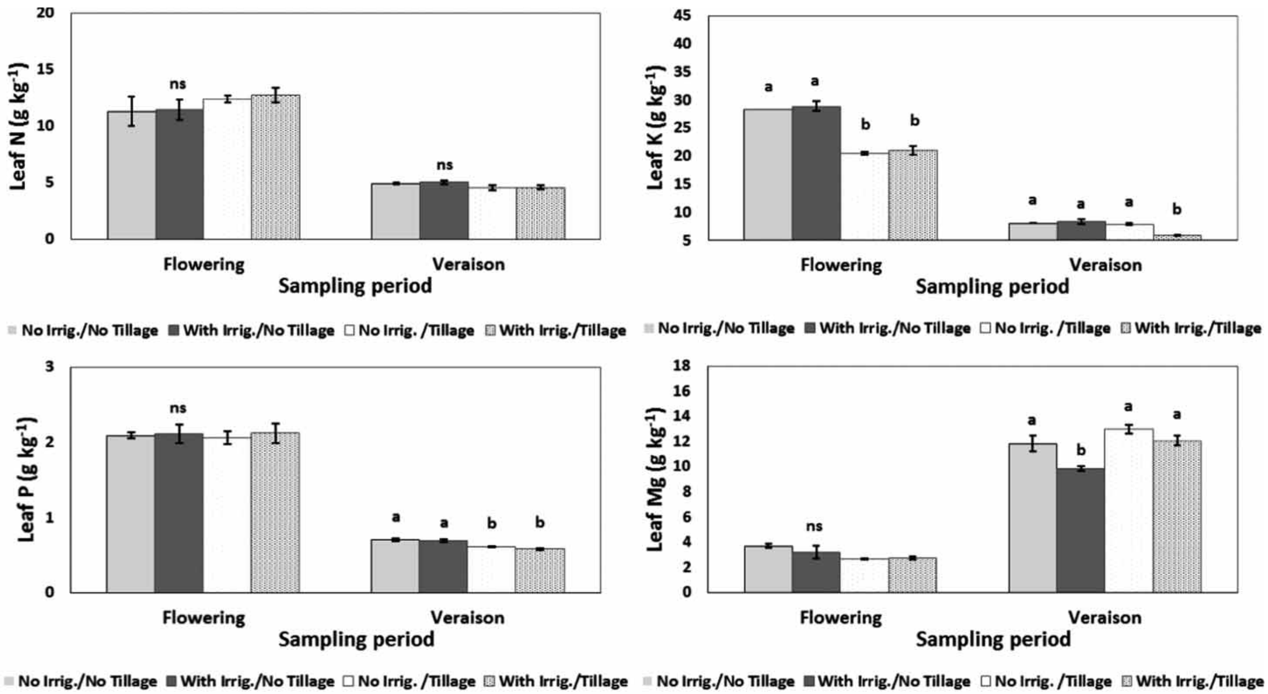

Figure 4 | Impact of irrigation and tillage on the leaf content of macronutrients. Sampling dates were during flowering (1st sampling) and veraison (2nd sampling). Significant differences ($P < 0.05$) among treatments are indicated by different letters according to Duncan's multiple range tests. Error bars show SE ($n = 4$).

Table 2 | Effects of irrigation and tillage on total soluble solids (TSS: °Brix), titratable acidity (TA: % tartaric acid), pH, ascorbic acid (AA: mg 100 mL^{-1} grape juice), total phenols (gallic acid equivalent: GAE 100 g^{-1} Fwt), anthocyanins (mg cyn-3-glu 100 g^{-1} Fwt) and tannins (mg 100 mL^{-1} grape juice)

		TSS	TA	pH	AA	Phenols	Anthocyanins	Tannins
No irrigation	No tillage	24.07 ± 0.38a	0.635 ± 0.028a	3.11 ± 0.04ab	0.81 ± 0.17a	5.33 ± 0.09b	90.25 ± 1.50a	43.49 ± 8.91b
	Tillage	22.37 ± 0.22b	0.636 ± 0.014a	3.14 ± 0.03ab	1.19 ± 0.32a	5.81 ± 0.09a	81.11 ± 4.49b	33.82 ± 2.16bc
Irrigation	No Tillage	20.50 ± 0.18c	0.646 ± 0.023a	3.05 ± 0.04b	1.02 ± 0.33a	4.09 ± 0.11c	62.01 ± 2.79c	66.04 ± 4.61a
	Tillage	22.85 ± 0.75ab	0.664 ± 0.028a	3.17 ± 0.02a	1.26 ± 0.17a	5.92 ± 0.18a	47.52 ± 0.35d	19.33 ± 3.52c

Values ($n = 4$) in columns followed by the same letter are not significantly different. $P \leq 0.05$ according to Duncan's multiple range tests.

months (June–September). Therefore, stomatal conductance is not affected by tillage or irrigation. As expected, the effect of drought was much more obvious at veraison (July), especially for non-irrigated vines. The plants during the hot and dry period reduce transpiration to adjust to the reduced availability of water. Irrigation under these conditions significantly increases transpiration and stomatal conductance. As the projections for the climate in Cyprus indicate increased drought and higher temperatures (Terink *et al.* 2013; Lelieveld *et al.* 2016), especially for the spring and summer months, we expect more severe drought stress in vines. Irrigation in such a case will be vital for the survival of the vines, and great effects are expected on plant physiology and yields, as shown in the results of the experiment for the various treatments (Figure 2).

Irrigation and tillage had no effect on chlorophyll content at the flowering and veraison stages while the content of chlorophylls (a, b, and total) was lower for the irrigation/no tillage treatment at the harvesting stage. In addition, less Mg was present in the leaves of the vines in the irrigation/no tillage treatment at veraison (Figure 4). Magnesium is essential for chlorophyll synthesis as Mg^{2+} is placed into protoporphyrin (Brzezowski *et al.* 2016). Therefore, less Mg in the leaves might result in reduced synthesis of chlorophylls in comparison to the other treatments.

The response of grapevines to moderate irrigation might also be cultivar-dependent as *V. vinifera* cultivars have been shown to respond differently to water stress (Schultz 1996). Grapevine cultivars have also been reported to adapt to water deficit by modifications of their morphological and anatomical characteristics, such as alterations in leaf area (Gómez del Campo *et al.* 2003), root/shoot ratio (Toumi *et al.* 2007), and xylem vessel size and conductivity (Lovisolo & Schubert 1998). Maratheftiko decreased stomatal conductance and altered plant metabolism during vegetative and reproductive stages. To promote adaptation to conditions of limited rainfall and less irrigation water, indigenous cultivars, well adapted to drought can be used (Koundouras *et al.* 2008). Maratheftiko is a promising cultivar for adapting to climate change and the mechanisms of adaptation to water shortage should be further explored.

There were no statistical differences in polyphenol content of leaves among treatments at the flowering stage, while differences were observed at veraison and harvesting stages, where heat and dry stresses were more intense. At veraison, tillage (independently of irrigation) increased total phenols in leaves and the same trend was observed at the harvesting stage, but the highest concentration was observed in the no irrigation/tillage treatment. Regarding antioxidant status, FRAP increased in the no irrigation/tillage treatment at the veraison stage, while at the harvesting stage it increased in all treatments. ABTS also increased at the harvesting stage and the higher value was observed for the no irrigation/tillage treatment but there were no differences in ABTS among the treatments in the other two sampling periods. Polyphenol synthesis (and antioxidant activity) is stimulated in plants in response to stress and phenolic compounds have an important role in plants' resistance to abiotic stress (Bettaieb *et al.* 2012). Drought (water deficit) is an abiotic stress and according to Abreu & Mazzafera (2005), the increase in polyphenols can be associated with an increase in secondary metabolites through the relocation of carbon, as plant growth is reduced. Drought stress in the roots could trigger oxidative stress in the whole plant because of changes in root absorption for both water and minerals. The stress might cause the aerial parts of plants to increase antioxidant substances as protection from injury by reactive oxygen species (Blokhina *et al.* 2003). However, in the case of Maratheftiko vines, it seems that tillage also plays an important role in polyphenols' content and resistance to abiotic stress. It is possible that tillage impacts the vine and triggers similar effects to the plant as water deficit does. The traffic and the weight of machinery used during tillage could affect or even injure the root system of vines as well as decrease the soil porosity (air- and water-filled pore space), and this could result in stress and the subsequent synthesis of antioxidant substances. In support of our findings are the results of Bahar & Yasasin (2010) where conventional and conservation tillage also increased the phenolic compounds in grape juice from Cabernet Sauvignon vines. We obtained similar results to those researchers for the Maratheftiko grapes' juice quality, where the highest phenolic content was in the tillage treatments (Table 2).

Vines accumulated more N, P, and K and less Mg in their leaves during flowering compared to veraison. At flowering, irrigation and no tillage led to increased K concentration in vine leaves. The nutrient concentration in leaves decreased at veraison and the lowest K concentration was observed for the irrigation/tillage treatment. A similar trend was also observed for P and its concentration in leaves also decreased at veraison when tillage was applied. The decreased K and P content in leaves during veraison for the irrigation/tillage treatment is related to the increased yield observed for that treatment, as minerals were mobilized for the berry development. The uptake of nutrients, such as P and K, which is very difficult to mobilize in soil and transfer to the soil solution, is promoted in vines as well as in other plants by mycorrhiza (Schreiner 2005). When mycorrhizas associate with a grapevine, the fungi help the vine increase its root network and its nutrient uptake by increasing the volume of soil explored. This increases grapevine growth and nutrition by better access to soil nutrients and by activating the regulation of plant transport proteins for P, N, K, and other elements (Trouvelot *et al.* 2015). Reduced water inputs and no tillage increase the presence of mycorrhizal hyphae in vineyard soil (Schreiner 2005). In addition, it was observed that tillage could mobilize nutrients such as P and K and make them available to the vines, and therefore indirectly affect mycorrhizal hyphae, which are not favored by elevated concentrations of nutrients in soils (Trouvelot *et al.* 2015). Considering the above, tillage and irrigation in the Maratheftiko vines reduced the uptake of P and K at veraison and this is probably due to disturbance of the mycorrhiza and reduction of the soil volume that the vine roots could utilize. However, such an observation needs to be studied further by soil microbiome and mycorrhiza presence research. Finally, less Mg was present in the leaves of the vines in the irrigation/cover crop treatment, at veraison. It is possible that the cover crop, especially when irrigated, utilized a part of the available Mg in the soil solution. Therefore, the concentration of the nutrient in the vine leaves was reduced in comparison to the other three treatments. This reduction was linked to the reduction of chlorophyll content in Maratheftiko leaves, as previously discussed.

Grape juice quality characteristics are very important for the production of high quality wines. TSS in grape juice increased in berries from vines receiving no irrigation/no tillage. This is in agreement with Bahar & Yasasin (2010) for Cabernet Sauvignon, where tillage reduced TSS in grape juice. Total phenols' content in grapes can increase under water stress (Deis & Cavagnaro 2013). This was observed in our research when comparing total phenols in irrigated and non-irrigated vines, but absence of tillage also plays a significant role in Maratheftiko (Table 2). Anthocyanin concentration is affected by environmental factors and biotic stresses and in the case of water stress their concentration can increase (Deis & Cavagnaro 2013). The trend was captured in our results for Maratheftiko. Anthocyanins were significantly higher in berries in the absence of either irrigation or tillage (Table 2). The increased TSS and anthocyanins and the decreased tannins' levels in the absence of irrigation and tillage suggest a speed up of metabolism related to berry ripening/maturation. During drought incidents, skin growth itself can be inhibited which could alter the proportion of skin and seeds to total berry weight, which determines tannins' concentration in the juice. Accordingly, in our study, irrigation and tillage significantly decreased tannins in the grape juice, leading to lower juice quality. Irrigation and cover cropping resulted in lower juice pH and no significant differences were observed in the content of ascorbic acid and in titratable acidity. As the projections for climate change in Cyprus indicate more frequent and severe drought, which has already resulted in reduced water availability for farmers in 2017–2018, it is possible that no irrigation will be more common in viticulture. In this case, no tillage might be useful as it leads to higher tannins' concentration in the Maratheftiko grape juice in comparison to tillage, which is important for the making of higher quality wines.

CONCLUSIONS

In this research, the effect of irrigation in combination with tillage was examined in the native Maratheftiko red cultivar. We showed that for the indigenous Maratheftiko variety, the implementation of no irrigation/no tillage systems might aid in adapting to climate change, since the irrigation/no tillage application did not increase yields. In addition to buffering yield losses, no irrigation/no tillage improves the quality characteristics of berries. With this study very useful information was obtained in designing soil management in particular and in grapevine cultivation under climate change in general. Additional research is required to explore the effects of tillage on soil microbiome and mycorrhizal function, an important issue for vine nutrition. These aspects have never been systematically studied for indigenous cultivars in Cyprus, which are more suited to the arid environment and are highly important for the island's winemaking sector. The research should expand to include additional indigenous varieties of the island, such as Xynisteri and Mavro. The results will be very useful for the adaptation of Cypriot and global viticulture to climate change.

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