BIOGEOSCIENCES AND WINE: THE MANAGEMENT AND ENVIRONMENTAL PROCESSES THAT REGULATE THE TERROIR EFFECT IN SPACE AND TIME

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EDITORIAL
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Editorial: Biogeosciences and Wine: The Management and Environmental Processes That Regulate the Terroir Effect in Space and Time

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Editorial on the Research Topic

Biogeosciences and Wine: The Management and Environmental Processes That Regulate the Terroir Effect in Space and Time

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Viticultural terroir is formally defined by the International Organization of Vine and Wine (OIV, 2010) as "*a concept which refers to an area in which collective knowledge of the interactions between the identifiable physical and biological environment and applied vitivinicultural practices develops, providing distinctive characteristics for the products originating from this area*". Though the OIV's definition does mention neither the time perspective inherent with terroir shaping nor its facet of cultural inheritance, which are important aspects, the study of terroir includes multidisciplinary approaches accounting for soil, geomorphology, morphometry, climate, as well as vineyard management, grapevine genotypes, historical know-how, and experiments, in addition to oenological practices (Deloire et al., 2005; Van Leeuwen and Seguin, 2006; Vaudour et al., 2015). However, time-rooted scientific questions about sustainability, the resilience of the vineyard system (Vaudour et al., 2017; Bonfante et al., 2018; Costantini et al., 2018), efficiency, and traceability have recently emerged. This Research Topic groups several innovative studies about these key issues and the approaches that were carried out for studying the terroir as a complex system, especially under climate change.

In the opinion paper, Brillante et al. critically examine the improper but popular use of the terroir concept, rejecting an implicit sensorial superiority for terroir wines and suggesting future directions for science in this field, with a focus on viticulture zoning. The authors stress the importance of characterizing and understanding the spatial variability of vineyards and the effects on grapevine physiology and grape composition independently from the price range of wines and connect the bio-physical study of terroir to precision viticulture.

The perspective from White summarizes the actual knowledge about the effects of soil characteristics on the grapevine, and then wine, concluding that most of these relationships are dependent on individual grape cultivars and individual sites. Therefore, site-specific studies, are needed to comprehend the soil effect on the specific wine. Soil health and microorganisms are the focus of the review from Lazcano et al., collecting the main results of studies on sustainable management practices, namely cover cropping and composting, in vineyards. The authors propose to include in the "terroir concept", the dynamic aspects of soil health driven by soil organic matter

and soil biota which may influence vine performance, and potentially affect wine quality. The paper of Mocali et al. moves one step forward in this direction, with the results from a case study in Chianti Classico (Italy) where two near plots, within a single vineyard, showed very similar soil physical and chemical features, but different wines. The authors were able to link wine composition to sulfur-oxidation genes in the soil and to highlight the potential role of sulfur metabolism as determining co-factor in the vineyard-scale variation of grape characteristics.

Such site-specific approach is also supported by the paper of Yu and Kurtural. The authors report a case study of mapping grapevine stem water potential (Ψ_{stem}) and soil apparent electrical conductivity (*ECa*) within a vineyard in California. They found a good relationship between the spatial variability of ECa and Ψ_{stem} and they tested selective grape harvest in two statistically different zones of the vineyard. The two zones produced wines characterized by different amounts and quality of anthocyanins. Similar results were also reported by the paper of Yu et al., which describes a two-year case of study in a different area and also assesses the effects of vineyard variability on wine composition. An alternative method to map grapevine water stress and delineate homogeneous zones for selective harvest is proposed by Brillante et al. The authors use the carbon stable isotope composition ($\delta^{13}C$) of grape juice at harvest to capture the spatial variability of physiological response at the vineyard scale and then compare the efficacy of separating grape composition with this zoning technique respect to zoning obtained with traditional measurements (pressure chamber) and modern sensors (soil ECa, canopy reflectance). This article also shows tight correlations between Ψ_{stem}, leaf gas exchange, and $\delta^{13}C$ across multiple varieties and vineyard regions.

Vineyard variability is generally investigated at the soil and plant level, but Bois et al. present a very original paper on mapping rainfall variability at a local scale using a dense rain-gauge network composed of 45 sensors over a 28 km^2 area. They used the rainfall data as an input variable in a soil water balance model to understand the impact of rainfall variability on water available to plants and showed how local rainfall might contribute to change in grapevine water status as large as 50% of the simulated regional water balance spatial variability. Considering the relevance of understanding the effect of climate change on terroir systems, De Resseguir et al. present a very high-resolution mapping of temperature at the local scale (19,233 ha) across six different seasons. Their maps show an amplitude of up to 10°C in a given day, and 320 degree days in the Winkler index in the Bordeaux area. They complement these maps with phenological observations to inform strategies for adaptation of plant material and cultural practices to local temperature variability and change. Relationships between temperature and grapevine phenology are also investigated by Merrill et al., with a focus on assessing the effect of high temperatures during flowering in a controlled-environment experiment with 50 varieties. Their research is combined with a review of the studies of controlled warming on winegrape varieties.

Another important aspect investigated in this RT is the use of plant biostimulants, addressed in the paper of Cirillo et al. These authors studied this rising and environmentally friendly practice on *Vitis vinifera* L. cv 'Aglianico' in southern Italy, investigating the possibility to apply a biostimulant derived from tropical plant extracts to improve the defenses of grapevine. Biostimulants mitigate possible negative effects of reducing the use of chemicals suitable for pest and disease management. Foliar applications of biostimulant appeared to induce a different response depending on the environmental factors and on the oxychloride copper dose distributed.

This RT also includes a research paper by Tescione et al. on the use of Sr isotopic ratio ($^{87}Sr/^{86}Sr$) as a geochemical tracer of white wines. The paper shows that the $^{87}Sr/^{86}Sr$ isotopic ratio of the geological substratum and soil is preserved in the must, and then in the wine, with no contribution given by the addition of bentonite and yeast using during the white wine-making process. Therefore it can be a promising method to trace the geographic provenance of white wines.

AUTHOR CONTRIBUTIONS

SP coordinated the editorial writing, all authors contributed to writing and revision, and approved the submitted version.

REFERENCES

Bonfante, A., Monaco, E., Langella, G., Mercogliano, P., Bucchignani, E., Manna, P., et al. (2018). A Dynamic Viticultural Zoning to Explore the Resilience of Terroir Concept under Climate Change. *Sci. Total Environ.* 624, 294–308. doi:10.1016/j.scitotenv.2017.12.035

Costantini, E. A. C., Castaldini, M., Diago, M. P., Giffard, B., Lagomarsino, A., Schroers, H.-J., et al. (2018). Effects of Soil Erosion on Agro-Ecosystem Services and Soil Functions: A Multidisciplinary Study in Nineteen Organically Farmed European and Turkish Vineyards. *J. Environ. Manage.* 223, 614–624. doi:10.1016/j.jenvman.2018.06.065

Deloire, A., Vaudour, E., Carey, V. A., Bonnardot, V., and Van Leeuwen, C. (2005). Grapevine Responses to Terroir: a Global Approach. *OENO One* 39, 149–162. doi:10.20870/oeno-one.2005.39.4.888

OIV (2010). Definition of Viticultural "Terroir". Resolution OIV/Viti 333/2010.

Van Leeuwen, C., and Seguin, G. (2006). The Concept of Terroir in Viticulture. *J. Wine Res.* 17 (1), 1–10. doi:10.1080/09571260600633135

Vaudour, E., Costantini, E., Jones, G. V., and Mocali, S. (2015). An Overview of the Recent Approaches to Terroir Functional Modelling, Footprinting and Zoning. *Soil* 1, 287–312. doi:10.5194/soil-1-287-2015

Vaudour, E., Leclercq, L., Gilliot, J. M., and Chaignon, B. (2017). Retrospective 70 Y-Spatial Analysis of Repeated Vine Mortality Patterns Using Ancient Aerial Time Series, Pléiades Images and Multi-Source Spatial and Field Data. *Int. J. Appl. Earth Observation Geoinformation* 58, 234–248. doi:10.1016/j.jag.2017.02.015

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The Value of Soil Knowledge in Understanding Wine Terroir

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There is an extensive literature on the role of soil physicochemical factors such as rate of water supply, N supply and soil temperature in wine terroir expression, especially for dry-grown vines. Other recent literature invokes the possibility of unique strains of the natural yeast *Saccharomyces cerevisiae* influencing must fermentations to produce distinctive aroma profiles in wines. Others suggest that the composition of the soil microbiome at particular sites can influence vine growth, fruit composition and wine characteristics to create a microbial terroir. Because terroir is a multifactor concept, no general quantitative relationships between one or more soil properties and the distinctive characteristics of wine from a particular site have been identified; rather a unique combination of soil factor values interacts with local climate, grape variety, vintage, canopy management, and winemaker technique to determine a site's terroir. However, with modern methods of sensing spatially referenced values of environmental and other variables at high resolution, terroirs can be mapped. This provides a platform for monitoring terroirs over time and recording how they respond to changes in environmental factors or to manipulations in the vineyard and winery.

Keywords: soil physics, soil chemistry, soil microbiome, soil variability, climate change, wine terroir

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INTRODUCTION

Wine terroir is a multifaceted concept that is recognized as embodying three broad categories of factors (Barham, 2003):

1. Natural factors that are associated with the environment (sometimes called "endowments").
2. Human factors – involving the use of techniques in the vineyard and winery that are confined to a particular region (sometimes referred to as "technologies").
3. Historical factors – reflecting widespread public knowledge of the wine coming from a region and recognition that this has been a long tradition.

Because terroir is such a broad concept, in this article I shall restrict my comments to the first category of factors. This is consistent with the view of van Leeuwen et al. (2016) that terroir is an ecosystem concept primarily incorporating the vine's interaction with its environment, a view shared by many scientists (e.g., Matthews, 2016) and respected wine writers (e.g., Goode, 2014; Hunt, 2015). With respect to the natural resources of a site, van Leeuwen and Seguin (2006) identified climate, soil and vine cultivar as the key factors that interact to determine the terroir. Although van Leeuwen et al. (2004) concluded, from a multi-season study in Bordeaux vineyards, that climate had the most significant effect on grape properties, van Leeuwen and de Rességuier (2018) acknowledged a specific effect of soil in terroir expression. While recognizing the

complexity of the interactions determining terroir, in this article I aim to update my review of the soil component (White et al., 2007), focusing on soil properties as they affect vine phenology and the consequential effects on the composition of grapes that are converted into wine. In so doing, I argue, along with van Leeuwen and de Rességuier (2018), that the recognition of terroir must be based on a wine's sensory characteristics that are consistently evident over a considerable period of time. Note that this requirement implicitly embraces the other two terroir categories suggested by Barham (2003); that is, an historical tradition that rests not only on consumer perceptions but also on the consistency of winemaking techniques.

CONCEPTS OF TERROIR

Amongst soil scientists, the concept of terroir most commonly accepted is that championed by the Bordeaux school (the Institut des Sciences de la Vigne et du Vin) (e.g., Seguin, 1986; van Leeuwen et al., 2004; van Leeuwen and Seguin, 2006; van Leeuwen and de Rességuier, 2018). In this, terroir is identified as an ecophysiological concept whereby a wine's sensory characteristics are related to the geographical origin of the wine. Two important consequences flow from this concept – first, that the appreciation of a wine's terroir depends on the consumers of the wine, whether they be experienced tasters or individuals not concerned with the origin of the product, only its enjoyment. Secondly, as Seguin (ibid.) explained, the geographical "fingerprint" of a wine explicitly or implicitly invokes the influence of many factors, which he listed as:

- Climatic conditions.
- The heat and light conditions of the vine's microclimate resulting from the particular training system.
- The cultivar and rootstock.
- The yield.
- Topography.
- Soil properties, including the water supply and mineral nutrition, especially of nitrogen (N).
- The "ecogeopedological milieu."

To this, Seguin (ibid.) added technological factors relating to winemaking techniques and conservation of the wines. Aside from the technological factors, this long list of ecophysiological factors is a fertile field for researchers to investigate which, if any, of these factors alone or in combination is definitive in determining the terroir of a wine from within a vineyard block, or an individual vineyard, or a region. Seguin did not mention soil biological factors, except as may be covered by the term "ecogeopedological milieu." However, the influence of the soil microbiome leading to the concept of a microbial terroir has received considerable attention in recent years, as is discussed later (Gilbert et al., 2014).

At the time of writing, Seguin (ibid.) discussed wine produced from vineyards in Bordeaux appellations with little reference to the scale at which resource factors might vary. However, the issue of scale became important as the terroir concept was extended to other French regions, and globally. For example, Vaudour et al. (2015) reviewed the tools available for rapid proximal- and remote-sensing of soil properties, coupled with the storage and manipulative power of geographic information systems, to map viticultural terroirs at a range of scales. Proffitt et al. (2006) demonstrated how the systematic analysis of spatial variability within Australian vineyards could be used not only to define terroirs, if that was a vigneron's objective, but also how this knowledge could be used to direct soil and canopy management, pest and disease control and selective harvesting. van Leeuwen et al. (2010), in describing methods for viticultural zoning based on soil properties, emphasized the importance of having clear objectives whether they be the demarcation of production areas, or adaptation of management practices to soil type, or protection of viticultural landscapes. Predictably, the precision of zoning and hence the reliability of the information provided increases with scale, but so does the cost. Nevertheless, as Vaudour et al. (2015) have noted, terroir maps at an appropriate scale can be updated and hence used to monitor changes in the underlying variables in response, for example, to climate change. The information provided can then inform changes in viticultural management practices to offset or at least ameliorate the effects of such change.

PHYSICOCHEMICAL FACTORS – WATER, NITROGEN AND TEMPERATURE

van Leeuwen and de Rességuier (2018) have argued, from many detailed studies in dry-grown French vineyards, that water and nitrogen supply to the vines, as well as soil temperature, are the major soil determinants of the terroir effect. These authors discuss how moderate soil water deficits are necessary to produce high quality wines, especially for red varieties. They describe how this condition can be achieved through under-drainage in wet clay soils, by appropriate choice of variety/rootstock, or choice of vine training system in dry regions, or by deficit irrigation where irrigation is practised. Also discussed is how a controlled N supply prevents excessive vegetative growth, but that sufficient N must be supplied to generate adequate Yeast Available N in the fruit and promote the synthesis of flavor and aroma compounds (especially in white wines) (Choné et al., 2001). With respect to soil temperature, the authors state that optimal terroir expression depends on the timing of grape ripeness toward the end of a season. Although the timing of ripeness is mainly driven by air temperature, they argue that soil temperature, which can be affected by the nature of the soil surface, as well as land slope and aspect, is also important.

The implicit conclusion to be drawn from this analysis is that the action of these factors, singly or in combination, is sufficiently different among different sites for the wines produced at any one site to express a distinctive terroir. Van Leeuwen and de Rességuier's (ibid.) stated that once the key soil factors have been quantified, vignerons can choose their planting material and management practices accordingly, to optimize the terroir expression of a site. Among the management practices identified, they acknowledged that irrigation may be necessary in dry areas

to obtain economically sustainable yields, but stated that "only deficit irrigation …. is compatible with terroir expression." However, this opinion reflects the Bordeaux school's emphasis on soil water deficits and the rate of water supply as the predominant factors in a terroir effect, which is not necessarily borne out by research in irrigated vineyards. For example, Bramley et al. (2011) studied the grape and wine characteristics of a Cabernet Sauvignon vineyard in the Murray Valley, Australia (mean annual rainfall 289 mm), which received up to 5 ML/ha/year of irrigation from 2004 to 2007. In this vineyard of 8.2 ha, which was not deficit-irrigated, zones of high and low yield were consistently identified that produced wines with different sensory properties (color and phenolics), the causes of which could not be established. However, the authors deemed that these differences could reflect a terroir difference due to biophysical characteristics of the site other than water supply. A similar conclusion was reached in a study of irrigated Shiraz vines in the Grampians region, Victoria, Australia, as discussed below (Bramley et al., 2017).

Many vignerons have an empirical knowledge of the robustness, or otherwise, of the relationships between particular soil properties at a site and the composition of the grapes produced and wines made: what is lacking in most cases is a quantitative understanding of these relationships. For both dry-grown and irrigated vineyards, key questions to be asked are:

- What is the critical soil water content (or water potential) corresponding to a desirable stress level in the vines – at what point in the vine's phenology and for how long is this applicable and how are these criteria affected by soil water-holding capacity and depth?
- Can the optimum soil N supply during growth be defined in terms of soil mineral N available in the whole soil profile at bud burst, or in terms of soil organic N, or potentially mineralizable N (measured by laboratory incubation), which is found to be correlated with soil organic N (White et al., 2008)?
- What is the optimum range for soil temperature to express a site's terroir? Although van Leeuwen and de Rességuier (ibid.) cited the "ideal window" for ripeness to be March in the southern hemisphere and between 10 September and 15 October in the northern hemisphere, they gave no soil temperature criteria.
- Are there specific soil chemical or biological properties responsible for a terroir effect at a given site?

Because soil properties interact with many other environmental properties to determine vine growth and fruit composition, it is likely there is no generally applicable answer to these questions: rather the critical combination of factor values will depend on the grape variety (sometimes the clone) and vintage (Bodin and Morlat, 2006), canopy management (Kliewer and Weaver, 1971), and soil type (van Leeuwen et al., 2009), and therefore be different from site to site. This conclusion reinforces the concept that a distinctive wine terroir reflects the uniqueness of the interaction of variables at a given site.

PHYSICOCHEMICAL FACTORS – SOIL NUTRIENTS

In his seminal 1986 paper, Seguin wrote at the time that "it is impossible to establish any correlation between wine quality and the soil content of any nutrient element." (Seguin believed that the expression of terroir and wine quality were interdependent). van Leeuwen and de Rességuier (2018) reiterated this view. Nevertheless, Seguin (ibid.) also offered the somewhat contradictory notion that soils of the Premier Grand Cru Classé (First Growths) in Bordeaux were generally nutritionally "richer" than soils producing lesser wines, not because they were naturally that way, but because the vineyard owners had nurtured these soils over many years.

Subsequent to Seguin's statement about the role of nutrients, a range of sophisticated analytical techniques have been developed for measuring the content of nutrient elements and many non-essential elements in soil. Vaudour et al. (2015) reviewed examples of the use of selected methods for relating wine composition to soil or parent rock composition, from which it may be concluded that these methods have value for determining the provenance of a wine. However, even though in many cases authors invoked the concept of terroir (e.g., Imre et al., 2012; Tarr et al., 2013), their results did not reveal any relationship between a wine of distinctive character and the supply of an element in soil, whether it be an essential nutrient or otherwise. In the context of vine uptake, three main problems persist for the successful quantification of such a relationship:

- Variations in the bio-availability of a nutrient, as controlled by soil processes such as precipitation/dissolution, sorption/desorption, buffering, mass flow (influenced by water flow), diffusion and microbiological transformations.
- Variations in the demand for a nutrient created by the growing vine and the selectivity for individual elements imposed at the absorbing root surface and in translocation within the plant.
- The influence of local climate fluctuations on the aforesaid bio-availability and demand factors.

OTHER QUESTIONS

In addition to the questions raised above, arising from our lack of quantitative knowledge of soil property–terroir relationships, Seguin's comment about Bordeaux First Growths begs the question – to what extent can the many variations in vineyard management modify a site's terroir (this is discussed further under 'Microbiological factors)? Moreover, assuming one or more quantitative soil–terroir relationships can be demonstrated for a given site, how independent are such relationships of vintage variations caused by climatic variations from one season to another? A study of the distinctive character of Shiraz grapes in the Grampians region of Victoria provides a partial answer to this question. Although not specifically focused on soil properties, Scarlett et al. (2014) and Bramley et al. (2017) found that the variation within a single vineyard in the concentration of

rotundone, a grape compound responsible for the pepper aroma of some Shiraz wines (Jeffery et al., 2009), was spatially structured and closely related to the topography of the land. This effect was most likely due to the influence of aspect and slope on variations in ambient temperature and/or the amount of incident solar radiation received (Zhang et al., 2015). The pattern of variation was the same for three consecutive vintages, even though the mean rotundone concentration differed 40–fold between vintages. Thus, the terroir effect in the studied vineyard appeared to be temporally stable. Gupta et al. (2019) have investigated this vineyard further to determine whether differences in any soil properties, especially the structural composition of the microbiome, might be associated with the rotundone variations, as discussed later.

Finally, there is the question of how climate change might affect the recognized terroir of many famous vineyards in iconic wine-growing regions. Hannah et al. (2013) derived ensemble mean temperature projections for the year 2050 for world wine regions, based on 17 Global Circulation Models and two Representative Concentration Pathways. They forecast that the suitability of current viticultural areas would decrease by 17–85%, depending on the region. van Leeuwen et al. (2013) disputed this forecast, citing examples of premier regions in France and Germany that were sustaining high-quality viticulture in spite of higher temperatures. They pointed to methodological flaws in the climate modeling, which used uncapped Growing Degree Days (GDD), underestimated varietal tolerances of higher temperatures and relied on a monthly time-step in calculating GDD. Irrespective of questions about the modeling, the projected decreases in suitable area in some regions were so large that some viticultural changes must occur, even though as van Leeuwen et al. (ibid.) stated, growers are adapting through changed management to higher temperatures and greater water stress. However, these authors also referred to the "evolution of consumer's preferences," which suggests that terroir expression is as much in the nose and taste of the consumer as it is in the natural endowments of a vineyard.

MICROBIOLOGICAL FACTORS

Microbial terroir is a recent concept based on the idea there is a unique composition of the microbial population (the soil microbiome) at a site. The existence of diverse populations of microorganisms associated with grapevines in the rhizosphere and as endophytes and epiphytes has been known for some time, and the possible influence of particular populations on wine metabolites has been explored (reviewed by Liu et al., 2019). However, with the development of advanced genetic-based techniques, such as next generation sequencing, the focus of the research has been on (1) the uniqueness of the natural yeast population present in the soil and on various plant parts, and how this influences the array of compounds synthesized during fermentation, and (2) the uniqueness of the suite of microorganisms found in the soil and also on plant parts, which influence the sensory properties of the fruit and wine.

Noting that strains of *Saccharomyces cerevisiae* outcompeted other yeast strains during the later stages of alcoholic fermentation, Goddard (2010) reported that the natural populations of *S. cerevisiae* in New Zealand soils were genetically distinct from those found internationally. Subsequently, Knight et al. (2015) identified sub-populations of these organisms residing in each of six major New Zealand wine regions. They used a representative genotype from each regional sub-population to ferment Sauvignon Blanc juice and found there were differences in the aroma and flavor of the wines produced. The authors argued that their results showed there was a quantifiable microbial contribution to terroir, acknowledging that they could not say anything about the temporal stability of these results. The latter proviso is important because, given the potential transfer of the organisms through insects, for example, the suite of *S. cerevisiae* strains in a vineyard or sub-region in any 1 year may differ from that in other years. This requires further study to confirm the significance of a yeast contribution to microbial terroir.

The research of Bokulich et al. (2013) and others supports the hypothesis that soil microbial composition and activity are linked with wine terroir. For example, Burns et al. (2015) studied the structure of the soil microbial communities in 19 vineyards selected from sub-appellations of the Napa Valley American Viticultural Area (AVA). These sub-appellations, originally delineated by qualitative assessment of climatic, topographic and edaphic features (including soil), were found to have different soil microbial communities. However, the authors were undecided as to whether the differences were merely correlated with the AVA features, or the microbiota had a direct effect on vine growth and fruit properties. Similarly, Zarraonaindia et al. (2015) showed that the majority of bacterial communities associated with vine parts (leaves, flowers, fruit, and roots) were highly localized and reflective of the corresponding soil populations. Bokulich et al. (2016) provided a further link in the chain by showing that the microbial "fingerprint" derived from the vineyard and winery was reflected in the fermentations and correlated with the chemical composition of the finished wines. Nevertheless, as the authors indicate, correlation is not causation and more research is needed to link one or more functional characteristics of the soil microbiome to a specific sensory property of the finished wines. Gupta et al. (2019) (ibid.) reached a similar conclusion after they found distinct differences in the microbial communities in soils supporting high and low rotundone concentrations in the Grampians, Victoria; however, they could not assign any specific function to the few microbial taxa/groups that accounted for these differences.

Another group of soil microorganisms that have been extensively studied is the arbuscular mycorrhizal fungi (AMF), common symbionts with grapevines. Although much is known about the effect of AMF on nutrient uptake, especially phosphorus, zinc and copper (Schreiner, 2005), little is known about the creation of a recognized wine terror through an AMF–vine cultivar symbiosis. For example, based on experiments with a specific clone of Tempranillo inoculated with a commercial AMF inoculum (a mixture of five species), Torres et al. (2019) speculated that infection with AMF might enhance the amino

acid content of the grapes, which may in turn affect the aromatic characteristics of the wine.

CONCLUSION

Although factors influencing terroir such as soil water supply, N availability and soil temperature have been extensively reported in the literature, we still lack quantitative information on these relationships for individual grape cultivars at individual sites. Similarly, whereas the relationships between an existing soil microbiome, including the yeast *S. cerevisiae*, and vine phenology and grape composition have been explored in a variety of environments, quantitative relationships between a species or group of microorganisms and wine sensory properties have yet to be revealed. Gilbert et al. (2014) (ibid.) speculated that, with the modern genomic tools of microbial ecology, we may develop a mechanistic understanding of the practices that vignerons have used for centuries, even to the point where the soil microbiome could be manipulated to improve soil quality and wine terroir. Similarly, looking to the future, Vaudour et al. (2015) suggested that knowledge of the microbial genome could indicate how a soil could be manipulated by probiotics designed to select suitable bacterial species, which could improve soil quality and crop productivity. Echoing this speculation, Gupta et al. (2019) (ibid.) suggested that with such an understanding, a vineyard could be managed to achieve specific grape and wine attributes, for example, by inoculation with specific organisms and/or by particular management practices. Perhaps in the future a test of this speculation might involve characterizing the soil microbiome of long-established biodynamic vineyards compared with organic and conventional-managed vineyards in the same environment, to determine whether (a) significant differences existed and if so, were they were stable over time, and (b) were any differences reflected in the character of the wine. Given that our knowledge of the quantitative relationships between any of a site's natural endowments – physicochemical or microbiological above or below ground – and wine terroir is still in an exploratory stage, wine writers, sommeliers and vignerons will continue to rely on historical tradition and their sensory perceptions to define a wine's "sense of place." Nevertheless, as Ballantyne et al. (2019) pointed out, based on the views of experienced winemakers in three regions of the world, terroir is not a fixed concept, but is evolving under the influence of consumer preferences (van Leeuwen et al., 2013) and the impact of climate change on the functioning of the natural site and regional endowments.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

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REFERENCES

Ballantyne, D., Terblanche, N. S., Lecat, B., and Chapuis, C. (2019). Old world and new world wine concepts of terroir and wine: perspectives of three renowned wine makers. *J. Wine Res.* 30, 122–143. doi: 10.1080/09571264.2019.1602031

Barham, E. (2003). Translating terroir: the global challenge of the French AOC labelling. *J. Rural Stud.* 19, 127–138. doi: 10.1016/s0743-0167(02)00052-9

Bodin, F., and Morlat, R. (2006). Characterization of viticultural terroirs using a simple field model based on soil depth I. Validation of the water supply regime, phenology and vine vigour in the Anjou vineyard (France). *Plant Soil* 281, 37–54. doi: 10.1007/s11104-005-3768-0

Bokulich, N. A., Collins, T. S., Masarweh, C., Allen, G., Heymann, H., Ebeler, S. E., et al. (2016). Associations among wine grape microbiome, metabolome, and fermentation behavoir suggest microbial contribution to regional wine characteristics. *mBio* 7, 1–12. doi: 10.1128/mBio.00631-16

Bokulich, N. A., Thorngate, J. H., Richardson, P. M., and Mills, D. A. (2013). Microbial biogeography of wine grapes is conditioned by cultivar, vintage, and climate. *Proc. Natl. Acad. Sci. U.S.A.* 111, E139–E148. doi: 10.1073/pnas.1317377110

Bramley, R. G. V., Ouzman, J., and Boss, P. K. (2011). Variation in vine vigour, grape yield and vineyard soils and topography as indicators of variation in the chemical composition of grapes, wine and wine sensory attributes. *Austral. J. Grape Wine Res.* 17, 217–229. doi: 10.1111/j.1755-0238.2011.00136.x

Bramley, R. G. V., Siebert, T. E., Herderich, M. J., and Krstic, M. P. (2017). Patterns of within-vineyard spatial variation in the 'pepper' compound rotundone are temporally stable from year to year. *Austral. J. Grape Wine Res.* 23, 42–47. doi: 10.1111/ajgw.12245

Burns, K. N., Kluepfel, D. A., Strauss, S. L., Bokulich, N. A., Cantu, D., and Steenwerth, K. L. (2015). Vineyard soil bacterial diversity and composition revealed by 16S rRNA genes: differentiation by geographic features. *Soil Biol. Biochem.* 91, 232–247. doi: 10.1016/j.soilbio.2015.09.002

Choné, X., van Leeuwen, C., Cherry, P., and Ribereau-Gayon, P. (2001). Terroir influence on water status and nitrogen status of non-irrigated Cabernet Sauvignon (*Vitis vinifera*): vegetative development, must and wine composition. *S. Afr. J. Enol. Vitic.* 22, 8–15.

Gilbert, J. A., van der Lelie, D., and Zarraonaindia, I. (2014). Microbial terroir for wine grapes. *Proc. Natl. Acad. Sci. U.S.A.* 111, 5–6. doi: 10.1073/pnas.1320471110

Goddard, M. (2010). Australasian Science. *Microb. Terror.*

Goode, J. (2014). *The Science of Wine: From Vine to Glass*, 2nd Edn. Berkeley: University of California Press.

Gupta, V. V. S. R., Bramley, R. G. V., Greenfield, P., Yu, J., and Herderich, M. J. (2019). Vineyard soil microbiome composition related to rotundone concentration in Australian cool climate 'peppery' Shiraz grapes. *Front. Microbiol.* 10:1607. doi: 10.3389/fmicb.2019.01607

Hannah, L., Roehrdanz, P. R., Ikegami, M., Shepard, A. V., Shaw, M. R., Tabor, G., et al. (2013). Climate change, wine, and conservation. *Proc. Natl. Acad. Sci. U.S.A.* 110, 6907–6912. doi: 10.1073/pnas.1210127110

Hunt, M. (2015). *'Rebellion and the Meaning of Terroir', Jancis Robinson Purple.* Available at: http://www.jancisrobinson.com (accessed July 9, 2015).

Imre, S. P., Kilmartin, P. A., Rutan, T., Mauk, J. L., and Nicolau, L. (2012). Influence of soil geochemistry on the chemical and aroma profiles of Pinot noir wines. *J. Food Agric. Environ.* 10, 280–288.

Jeffery, D. W., Siebert, T. E., Capone, D. L., Pardon, K. H., van Leeuwen, K. A., and Solomon, M. R. (2009). Grape and wine pepper aroma – analytically challenging but we sniff it out in the end. *Tech. Rev.* 180, 11–16.

Kliewer, W. M., and Weaver, R. J. (1971). Effect of crop level and leaf area on growth, composition, and coloration of 'Tokay' grapes. *Am. J. Enol. Vitic.* 22, 172–177.

Knight, S., Klaere, S., Fedrizzi, B., and Goddard, M. R. (2015). Regional microbial signatures positively correlate with wine phenotypes: evidence for a microbial aspect to terroir. *Sci. Rep*. 5, 1–10. doi: 10.1038/srep14233

Liu, D., Zhang, P., Chen, D., and Howell, K. (2019). From vineyard to the winery: how microbial ecology drives regional distinctiveness of wine. *Front. Microbiol.* 10:2679. doi: 10.3389/fmicb.2019.02679

Matthews, M. A. (2016). *Terroir and Other Myths of Winegrowing*. Berkeley: University of California Press.

Proffitt, T., Bramley, R., Lamb, D., and Winter, E. (2006). *Precision Viticulture: A New Era in Vineyard Management and Wine Production*. Adelaide: Winetitles.

Scarlett, N. J., Bramley, R. G. V., and Siebert, T. E. (2014). Within-vineyard variation in the 'pepper' compound rotundone is spatially structured and related to variation in the land underlying the vineyard. *Austral. J. Grape Wine Res.* 20, 214–222. doi: 10.1111/ajgw.12075

Schreiner, R. P. (2005). "Mycorrhizas and mineral acquisition in grape vines," in *Soil Environment and Vine Mineral Nutrition*, eds L. P. Christensen, and D. R. Smart, (Davis CA: American Society of Enology and Viticulture), 49–60.

Seguin, G. (1986). 'Terroirs' and pedology of wine growing. *Experientia* 42, 861–873. doi: 10.1007/bf01941763

Tarr, P. T., Dreyer, M. L., Athanas, M., Shahgholi, M., Saarloos, K., and Second, T. P. (2013). A metabolomics based approach for understanding the influence of terroir in *Vitis vinifera* L. *Metabolomics* 9, S170–S177.

Torres, N., Hilbert, G., Antolín, M. C., and Goicoechea, N. (2019). Amino acids and flavonoids profiling in Tempranillo berries can be modulated by the arbuscular mycorrhizal fungi. *Plants* 8, 400–415. doi: 10.3390/plants8100400

van Leeuwen, C., and de Rességuier, L. (2018). Major soil-related factors in terroir and vineyard siting. *Elements* 14, 159–165. doi: 10.2138/gselements.14.3.159

van Leeuwen, C., Friant, P., Choné, X., Tregoat, O., Koundouras, S., and Dubourdieu, D. (2004). Influence of climate, soil and cultivar on terroir. *Am. J. Enol. Vitic.* 55, 207–217.

van Leeuwen, C., Roby, J.-P., and de Rességuier, L. (2016). "Understanding and managing wine production from different terroirs," in *Proceedings of the 11th International Terroir Congress*, eds G. V. Jones, and N. Doran, (Ashland: Southern Oregon University), 388–393.

van Leeuwen, C., Roby, J.-P., Pernet, D., and Bois, B. (2010). Methodology of soil-based zoning for viticultural terroirs. *Bull. l'OIV* 83, 13–29.

van Leeuwen, C., Schultz, H. R., de Cortazar-Atauri, I. G., Duchêne, E., Olat, N., Pieri, P., et al. (2013). Why climate change will not dramatically decrease viticultural suitability in main wine producing areas by 2050. *Proc. Natl. Acad. Sci. U.S.A.* 110, E3051–E3052.

van Leeuwen, C., and Seguin, G. (2006). The concept of terroir in viticulture. *J. Wine Res.* 17, 1–10. doi: 10.1080/09571260600633135

van Leeuwen, C., Tregoat, O., Choné, X., Bois, B., Pernet, D., and Gaudillère, J.-P. (2009). Vine water status is a key factor in grape ripening and vintage quality for red bordeaux wine. How can it be assessed for vineyard management purposes? *J. Int. Sci. Vigne Vin* 43, 121–134.

Vaudour, E., Costantini, E., Jones, G. V., and Mocali, S. (2015). An overview of the recent approaches to terroir functional modelling, footprinting and zoning. *Soil* 1, 287–312. doi: 10.5194/soil-1-2 87-2015

White, R. E., Balachandra, L., Edis, R., and Chen, D. (2007). The soil component of terroir. *J. Int. Sci. Vigne Vin* 41, 9–18.

White, R. E., Balachandra, L., Edis, R., and Chen, D. (2008). "Grapevine vigour is correlated with N-mineralization potential of soil from selected cool climate vineyards," in *Proceedings VIIth International Terroir Congress*, (Nyon: Agroscope Changins-Wädenswil).

Zarraonaindia, I., Owens, S. M., Weisenhorn, P., West, K., Hampton-Marcell, J., Lax, S., et al. (2015). The soil microbiome influences grapevine associated microbiota. *mBio* 6, 1–10.

Zhang, P., Barlow, S., Krstic, M., Herderich, M., Fuentes, S., and Howell, K. (2015). Within-vineyard, within-vine, and within-bunch variability of the rotundone concentration in berries of *Vitis vinifera* L. cv. Shiraz. *J. Agric. Food Chem.* 63, 4276–4283. doi: 10.1021/acs.jafc.5b 00590

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Proximal Sensing of Soil Electrical Conductivity Provides a Link to Soil-Plant Water Relationships and Supports the Identification of Plant Water Status Zones in Vineyards

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The majority of the wine grapes are grown in Mediterranean climates, where water is the determining factor for grapevine physiology and berry chemistry. At the vineyard scale, plant water status is variable due to the variability in many environmental factors. In this study, we investigated the ecophysiological variability of an irrigated Cabernet Sauvignon (*Vitis vinifera* L.) vineyard. We used equidistant grid sampling to assess the spatial variations of the plants and soil, including plant water status by stem water potential (Ψ_{stem}), leaf gas exchange, and on-site soil analysis. We also measured soil electrical conductivity (EC) by proximal sensing at two depths [0.75 – 1.5 m (sub soil); 0 – 0.75 m (top soil)]. Ψ_{stem} integrals were calculated to represent the season-long plant water status. On the base of realized Ψ_{stem} integrals, the vineyard was delineated into two functional homogeneous zones (fHZs) with one severely water stressed zone and one moderately water stressed zone. Sub soil EC was directly related to Ψ_{stem} (r^2 = 0.56) and g_s (r^2 = 0.39) when the soil was proximally sensed at harvest in 2018. Although the same trend was evident in 2019 we could not deduce a direct relationship. The fruits from the two fHZs were harvested differentially. Comparing the two fHZs, there was no significant difference in juice total soluble solids or pH. The severely water stressed zone showed significantly higher malvidin and total anthocyanins on a dry skin weight basis, but lower peonidin, malvidin on a per berry basis in 2018. In 2019, there were more quercetin and total flavonols per berry in the severely water stressed zone. Overall, this study provided fundamental knowledge of the viability of managing spatial variability by delineating vineyard into distinct zones based on plant water status, and the potentiality of proximally sensed soil EC in the spatial assessment of plant water status and the supporting of vineyard management.

Keywords: plant water status, soil electrical conductivity, spatial variability, selective harvest, anthocyanins, precision viticulture

INTRODUCTION

Plant water status is one of the major drivers affecting grapevine physiology (Smart and Coombe, 1983), and is a determinant of grape berry chemistry (Martínez-Lüscher et al., 2014a). When soil water availability cannot fully meet the needs of plant growth and development, it becomes an abiotic stressor of the plants. Many physiological processes are affected when plants undergo

water stress. The overall plant growth will be induced into reproductive maturity and dormancy as opposed to vegetative growth by the upregulation in abscisic acid synthesis (Chaves et al., 2010; Tombesi et al., 2015; Bonfante et al., 2017). For grapevines specifically, water stress was shown to influence canopy development, canopy microclimate, yield, and berry composition (Santesteban et al., 2011; Escalona et al., 2015). It would decrease leaf stomatal conductance and net carbon assimilation, leading to a decline in photosynthetic output. By various agronomic practices, water stress can be controlled within a mild to moderate range in red skinned wine grape cultivars. This may have beneficial effects on berry chemistry because water stress would suppress the grapevine vegetative growth being as a competing process for limiting photosynthetic resources (Intrigliolo and Castel, 2010). The assimilated carbohydrates are then repartitioned into berries and thus increasing total soluble solids (TSS) in the berries under moderate water stress, favoring the reproductive growth of the grapevines.

Vineyard systems are not uniform due to the existing spatial variability in growing site topography and soil characteristics (Brillante et al., 2017). Furthermore, cultural practices are usually applied uniformly without taking into account the spatial variability in vineyards. Besides, the complexity in vineyard systems makes it challenging to individualize each of the existing spatial variability for making management decisions. More often than not this leads to variability in grape composition at harvest, where the composition of the final wine would be compromised (Bramley, 2005). Thus, there is a need for a more comprehensive and precise approach to assess, monitor, and manage these variabilities by treating the vineyard as a whole system.

Proximal sensing in precision viticulture may be used to assess and monitor the spatial and temporal variability to fulfill this need (Matese et al., 2015). In a vineyard, under the same climate condition, the processes involved in the soil-plant continuum and the atmosphere system are strictly influenced by the soil spatial variability. These would unavoidably lead to a spatial variability in plant water status and berry composition (Brillante et al., 2014, 2018; Tardaguila et al., 2018). Assessing soil has been investigated in previous precision viticulture studies (Gómez-Míguez et al., 2007; Costantini et al., 2010; Bonfante et al., 2011; Brillante et al., 2017). Electrical conductivity (EC) (or its reciprocal electrical resistivity) was used to assess many soil variables as it acts as a function of soil physical and chemical properties, such as soil texture, moisture content, solute concentration, and temperature (Bushman and Mehalick, 1989; Bittelli, 2011). Proximal soil sensing is rapid and non-invasive, which can be utilized as a tool for soil assessments. Many studies have implemented electromagnetic induction (EMI) sensing in their data acquisition methods besides some other approaches such as ground-penetrating radar (GPR), and time domain reflectometry (TDR). This approach was shown the capability to capture the integrated effect of soil moisture, soil salinity and soil texture, and their spatial and temporal variability with a relatively high temporal resolution and promptness in data acquisition (Hardie and Doyle, 2012; Brillante et al., 2014; Su et al., 2014). The ability to assess soil properties and plant water status more rapidly by proximal sensing is beneficial in commercial vineyards because it can provide the possibility to monitor and manage the spatial variability in soil responsively, that may further minimize the variations in final berry composition and wine chemistry (Brillante et al., 2018). Additionally, due to the significance of plant water status on berry chemistry, it is possible to evaluate the berry chemical composition once the relationship between soil electromagnetic properties and plant water status is determined.

For wine grape cultivars, flavonoid compounds constitute the most abundant class of berry secondary metabolites. They are critical in determining organoleptic properties in wine, such as color, flavor, mouth-feel, and also aging potential (Lorrain et al., 2013). The biosynthesis of these compounds are responsive to plant water status, where moderate water stress usually resulted in upregulation in flavonoid biosynthesis (Castellarin et al., 2007a). Managed water stress can contribute to a higher ratio of tri-hydroxylated over di-hydroxylated flavonoids due to the up-regulation of flavonoid 3′5′-hydroxylases (F3′5′H) (Castellarin et al., 2007b), which would enhance the compound stability against degradation (Liu et al., 2018). However, the higher concentration of flavonoids observed under water stress may be due to berry dehydration rather than alteration of flavonoid biosynthesis (Hardie and Doyle, 2012). Based on the captured variability in vineyards, vineyard delineation can be utilized to minimize the variability between zones pairing with targeted agronomic practices (González-Fernández et al., 2017). Selective harvest is one example of these practices when fruits are picked differentially, or segregated into various batches prior to the fermentation for producing wine with different rankings or characteristics (Bramley et al., 2011b; Priori et al., 2019). This approach can coalesce the variable ripening stages that may occur within the vineyard, where the relatively unripe fruits would be imparting unripe characteristics in the final wines (Parr et al., 2007). The variability in grape productivity and composition will always be present to a certain extent within vineyards. However, some prevailing faulty characters due to the uneven ripeness stages resulting from the heterogeneity in vineyards may impair quality, yielding undesired sensory properties in the final products (Kontoudakis et al., 2011). Hence, it is necessary to be able to minimize the variability within vineyards to achieve relatively the same maturity for vinification, and selective harvest can provide a direct way to satisfy this purpose.

Based on these previous studies, the objectives of this study were to investigate the variability observed in soil EC, and how it is translated into the variability in plant water status. Subsequently, this study investigated the relationship between proximal soil sensing and grape berry chemistry to bridge the gap between available sensing technologies and advanced chemical analysis methods. We also investigated whether the selective harvest approach, by delineating vineyard into different management zones based on plant water status, would minimize the variability in grape berry chemistry; and whether this zoning can be directed by proximal soil sensing, specifically by soil EC.

MATERIALS AND METHODS

Vineyard Site, Plant Materials, and Weather

This study was conducted in a commercial vineyard with Cabernet Sauvignon grafted on 3309C (*V. riparia* × *V. rupestris*) in 2018 and 2019. This vineyard was located in Oakville, Napa County, California, United States. Grapevines were planted at 1.5 m × 2.0 m (vine × row), and trained as a bi-lateral cordon on a single high wire. The vineyard was pruned mechanically to a spur height of 100 mm with no further canopy management in 2018, and treated with mechanical shoot removal at E-L stage 17 to meet production demands. Irrigation was applied with a drip irrigation system with two 2L/h emitters at each plant, starting at fruit-set to harvest to replace 50% of crop evapotranspiration demand (ETc).

Weather data at the research site during the growing season was obtained from the California Irrigation Management Information System (CIMIS) station #77, in Oakville, CA which was 200 m away from the research site. Precipitation and reference evapotranspiration data were acquired to direct irrigation scheduling during the growing season. Applied irrigation amounts were calculated as the product of calculated crop coefficient and reference evapotranspiration. The crop coefficient was calculated as reported by Williams and Ayars (2005). Air temperature was acquired from the station for growing degree days (GDD) calculation.

Experimental Design

We used an equidistant 30 m × 30 m grid to sample and collect on-site measurements which contained 14 experimental units with 3 vines in each experimental unit. Geolocations of each center vine within each experimental unit were recorded with GPS unit (Yuma 2, Trimble Inc., Sunnyvale, CA, United States) connected to a Trimble Pro 6T DGNSS receiver (Trimble Inc., Sunnyvale, CA, United States) for further GIS analysis (**Figure 1**).

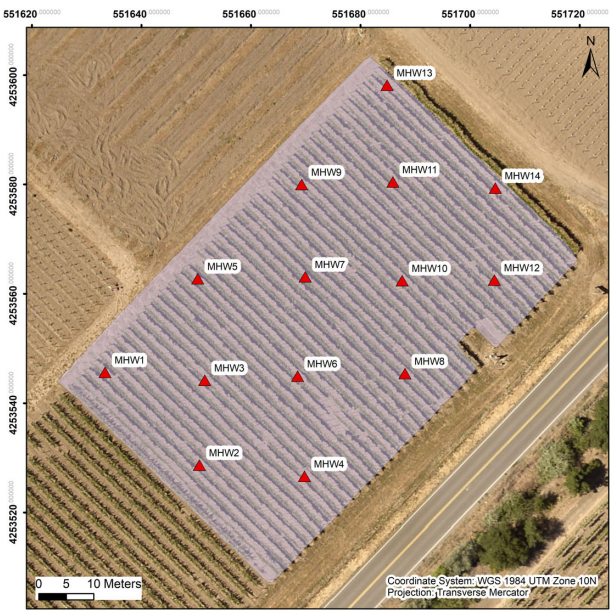

FIGURE 1 | Map of the experimental block with 14 experimental units marked, red triangles illustrate the locations of the middle vine among the three vines in each experimental unit. "MHW" is the abbreviation for "mechanical high wire", as named for the experimental block.

Soil Property Assessment

Soil samples were taken on at field capacity from the two depths in the 14 experimental units, corresponding to the two depths that proximal soil sensing was conducted. Soil total organic matter (OM) and soil texture were measured according to the soil analysis methods in the North American Proficiency Testing (NAPT) program, Western states section. OM was measured by loss on ignition method (S – 9.10), soil texture was acquired by hydrometer analysis (S – 14.10). Soil gravel content was determined by section 26 in USDA Handbook No. 60 (Diagnosis and Improvement of Saline and Alkali Soils).

The instrument used for assessing bulk soil electrical conductivity was EM38-MKII (Geonics Ltd., Mississauga, ON, Canada) used in both vertical dipole mode and horizontal dipole mode to assess two depths [0.75 – 1.50 m (sub soil EC) and 0 – 0.75 m (top soil EC)] of measurements. The sensor of the instrument was calibrated according to the manufacturer's instructions to minimize the errors before the survey. The instrument was placed on a PVC sled at an approximately 15 cm height above the ground, and pulled by an all-terrain vehicle along the inter-rows at a distance of about 0.5 m to avoid interference phenomena with the vehicle. The PVC sled made possible to keep the instrument at a constant distance from the soil surface, making data acquisition easier and more accurate. In both years, the soil EC measurements were assessed on the dates close to harvest, which occurred on 28 September 2018 and 20 September 2019.

Plant Water Status, Gas Exchange and Yield Component Assessment

Mid-day stem water potential (Ψ_{stem}) measurements were taken in 2018 and 2019 to assess plant water status. The measurements was assessed bi-weekly from 16 August 2018 and 29 May 2019. Three leaves in the shade were selected from the main shoot axis on the grapevines, and were concealed in pinch-sealed Mylar® bags for about 2 h prior to the measurements in each experimental unit. A pressure chamber (Model 615D, PMS Instrument Company, Albany, OR, United States) was used to take the measurements. To summarize the temporal information assessed by Ψ_{stem} measurements, Ψ_{stem} integrals were calculated by using natural cubic splines (Myers, 1988). The sum of the values were divided by the number of the days between the first and the last measurements in each year to make the data comparable to each individual measurement.

In parallel with Ψ_{stem} measurements at mid-day, leaf gas exchange measurements were taken to assess leaf photosynthetic activities by using a portable infrared gas analyzer CIRAS-3 (PP Systems, Amesbury, MA, United States). The measurements

were assessed bi-weekly from 16 August 2018 and 29 May 2019. Three sun-exposed leaves were selected from the main shoot axis in each experimental unit, and three readings were taken from each leaf. Gas exchange measurements were taken when the sunlight condition was close to saturating in both years (average $PARi$ = 1713 ± 249 μmol m^{-2} s^{-1} in 2018, 1721 ± 206 μmol m^{-2} s^{-1} in 2019). The relative humidity was set at 40%, the reference CO_2 concentration was set at 400 μmol CO_2 mol^{-1} as the standard environmental condition setting in CIRAS-3. Net carbon assimilation rate (A_N, μmol CO_2 m^{-2} s^{-1}) and stomatal conductance (g_s, mmol H_2O m^{-2} s^{-1}) were obtained. Intrinsic water use efficiency (WUEi) was calculated as the proportion of A_N over g_s (μmol CO_2 $mmol^{-1}$ H_2O). The g_s integrals were calculated to represent the long-term stomatal responses.

Leaf area index (LAI) was measured to characterize grapevine canopy growth, and converted into leaf area on 16 August 2018 and 15 August 2019 by a smartphone based program, VitiCanopy, coupled with an iOS system (Apple Inc., Cupertino, CA, United States) (De Bei et al., 2016). The gap fraction threshold was set to 0.75, the extinction coefficient was set to 0.7, and sub-divisions were 25. A 'selfie-stick' was used for easy access to place the device about 75 cm underneath the canopy. The device was positioned with the maximum length of the screen being perpendicular to the cordon, and the cordon being at the middle of the screen according to the user's instruction (De Bei et al., 2016). In each experimental unit, three images were taken to capture half canopy of each vine, and analyzed by the software. Total leaf areas were calculated based on both LAI values and unit ground area in each experimental unit, and then the leaf area to fruit ratio was calculated.

All clusters in each experimental unit were harvested, counted, and weighed on a single harvest day in both seasons (27 September 2018 and 23 September 2019). Yield components were then calculated for assessing cluster number per vine, average cluster weight, berry number per vine, and yield per vine. Single berry weight was calculated by averaging total berry weights by total berry numbers from the collected berry samples.

Berry Primary Metabolite Assessment

From each experimental unit 75 berries were randomly sampled, and were separated into two subsets with 55 berries and 20 berries individually. The set with 55 berries was used for berry primary metabolite analysis, including TSS, juice pH, titratable acidity (TA), and berry weight assessments. The set with 20 berries was for assessing dry berry skin weight and skin flavonoid contents.

Berry TSS was measured by a digital refractometer (Atago PR-32, Bellevue, WA, United States) and expressed as °Brix. Juice pH and TA were measured with an automated titrator (862 Compact TitroSampler, Metrohm, Switzerland) and expressed as g of tartaric acid per L of juice.

Extraction of Skin Flavonoid Compounds

Skin tissues were manually removed from the subset of 20 berries with a scalpel, separated from the seeds and pulps, and lyophilized (Centrivap Benchtop Centrifugal Vacuum Concentrator 7810014 equipped with Centrivap −105°C Cold Trap 7385020, Labconco, Kansas City, MO, United States). Dry skin weights were recorded after lyophilization, and then the skin tissues were powderized with a mixing mill (MM400, Retsch, Mammelzen, Germany). We used 50 mg (±5% deviation allowed) of dry skin powder and mixed with 1 mL of methanol:water:7 M hydrochloric acid (70:29:1) to initiate the extraction at 4°C for 24 h. Then, the extracts were centrifuged at 5,000 rpm for 15 min, and the supernatants were separated from the sediments, filtered by PTFE membrane filters (diameter: 13 mm, pore size: 0.45 μm, VWR, Seattle, WA, United States), and transferred into HPLC vials before injection.

Berry Skin Flavonoid Analysis

Skin anthocyanins and flavonols were analyzed by a reversed-phase HPLC (Agilent model 1260, Agilent Technologies, Santa Clara, CA, United States) consisting of a vacuum degasser, an autosampler, a quaternary pump, and a diode array detector with a column heater. A C18 reversed-phase HPLC column (LiChrosphere 100 RP-18, 4 × 520 mm^2, 5 μm particle size, Agilent Technologies, Santa Clara, CA, United States) was used for the utilized method. The mobile phase flow rate was 0.5 mL min^{-1}, and two mobile phases were used, which included solvent A = 5.5% aqueous formic acid; solvent B = 5.5% formic acid in acetonitrile. The HPLC flow gradient started with 91.5% A with 8.5% B, 87% A with 13% B at 25 min, 82% A with 18% B at 35 min, 62% A with 38% B at 70 min, 50% A with 50% B at 70.01 min, 30% A with 70% B at 75 min, 91.5% A with 8.5% B from 75.01 min to 90 min. The column temperature was maintained at 25°C. Detection of flavonols and anthocyanins was carried out by the diode array detector at 365 and 520 nm, respectively. A computer workstation with Agilent OpenLAB (Chemstation edition, version A.02.10) was used for chromatographic analysis.

All solvents used in this analysis were of HPLC grade, including acetonitrile, methanol, hydrochloric acid, formic acid purchased from Fisher Scientific (Santa Clara, CA, United States). Standards used for compound identification included malvidin 3-*O*-glucoside purchased from Extrasynthese (Genay, France), myricetin-3-*O*-glucuronide, myricetin 3-*O*-glucoside, quercetin 3-*O*-glucunoride, quercetin 3-*O*-galactoside, quercetin 3-*O*-glucoside, kaempferol 3-*O*-glucoside, isorhamnetin 3-*O*-glucoside, and syringetin 3-*O*-glucoside purchased from Sigma-Aldrich (St. Louis, MO, United States).

Statistical Analysis

Geostatistical analysis and kriging for soil EC were performed by using package gstat 1.1-6 (Pebesma, 2004). Due to the nature of proximal sensing, there were many outliers captured when assessing soil EC. The data were filtered by Tukey's rule to remove outliers of soil EC either below the first quartile by 1.5 inter-quartile range, or above the third quartile by 1.5 inter-quartile range. To further remove the outliers, the data were filtered by the speed that the vehicle was driving, which was between 3.2 km per hour to 8 km per hour. Variograms were assessed by automap package 1.0-14 (Hiemstra, 2013), and fitted to perform kriging. The specific soil EC values were extracted from the location of each experimental unit, these EC values were further used to performance correlation analysis.

Kriging was performed in ArcGIS (version 10.6, Esri, Redlands, CA, United States) and *k*-means clustering was performed in R (RStudio, Inc., Boston, MA, United States) with package NbClust, v3.0 (Charrad et al., 2014). An ordinary kriging method was used since there was no trend observed in the vineyard. In 2018, a spherical semivariogram model was chosen with a major range of 70.963 m, a nugget of 0, and a partial sill of 0.039 after cross-validation. The cross-validation of the model showed a root mean square error (RMSE) of 0.132 MPa, an average standard error of 0.124 MPa. In 2019, the vineyard was delineated into two clusters as well. A spherical semivariogram model was chosen with a major range of 35.48 m, a nugget of 0, and a partial sill of 0.012 after cross-validation. The cross-validation of the model showed a root mean square error (RMSE) of 0.100 MPa, an average standard error of 0.100 MPa. *k*-means clustering analysis and the practical manageability were considered when delineating the vineyard. The vineyard was delineated into two clusters by *k*-means clustering based on Ψ_{stem} integrals based on its significant role in connecting soil to plant physiology, including a severely water stressed zone and a moderately water stressed zone. The separation described 70.8% in 2018 and 67.8% in 2019 of the variability in the plant water status according to the result of between sum of squares/total sum of squares. Based on this delineation, data from the experimental units finally grouped together according to their locations within each cluster for the statistical analysis comparing grapevine physiological and berry chemistry measurements.

Data were tested for normality by using Shapiro–Wilk's test, and subjected to mean separation by using one-way ANOVA with the package "stats" in Rstudio (R Foundation for Statistical Computing, Vienna, Austria) (R Core Team, 2019). Significant statistical differences were determined when *p*-values acquired from ANOVA were of 0.05 or less. Linear regression analysis was performed by SigmaPlot 13.0 (Systat Software Inc., San Jose, CA, United States). The coefficient of determination between variables was calculated in linear regression analysis, *p*-values were acquired to present the significances of the linear fittings.

RESULTS

Weather and Soil EC at Experimental Site

During the execution of the experiment, the precipitations received during the 2 years were vastly different (**Figure 2**). The experiment site received 356.2 mm and 1132.1 mm precipitation (from the previous November to October when fruits were harvested) in 2018, and 2019 respectively. Of the total precipitations received 88.71% of them were received during the dormant season in 2018 (from previous November to April). In 2019, 91.98% of the precipitation received was during the dormant season. The precipitation during the growing season was very limited. The research site only received 0.5 mm in 2018 and 1.7 mm in 2019 during the study time in each year from June to September. GDD accumulation showed that in 2019, the heat accumulation was higher in the second year with 1668.9°C compared to 1521.7°C in 2018.

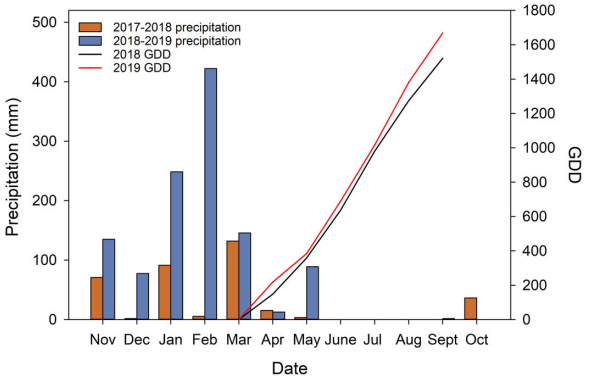

FIGURE 2 | Weather data acquired from California Irrigation Management Information System (CIMIS) station (#77 Oakville, CA) and calculated growing degree days (GDD).

Soil EC was assessed at two different depths close to harvest in both years. In 2018, EC values in sub soil were generally lower with the lowest value at 0.07 dS/m in the southwestern section as well as the central section of the vineyard, the rest of the vineyard having higher EC with the maximum value of 0.56 dS/m (**Figure 3A**). Top soil EC values in the southwestern section of the vineyard in 2018 with 0.42 dS/m compared to 0.43 dSm in the rest of vineyard and did not vary appreciably in this year (**Figure 3B**). However, EC values in sub soil were lower only in the central section of the vineyard in 2019, showing the lowest value of 0.15 dS/m and highest of 0.34 dS/m in the rest of the vineyard (**Figure 3C**). A similar trend was evident in the top soil in 2019 compared to the first season, where lower EC values of 0.07 dS/m were observed in the southwestern section of the vineyard compared to 0.16 dS/m in the rest of the vineyard (**Figure 3D**).

Plant Water Status, and Photosynthetic Activity

Ψ_{stem} was measured throughout both seasons, and the overall trend in long-term Ψ_{stem} was able to partially elucidate the trend seen in the soil EC maps. Two clusters were calculated within the vineyard to delineate the whole block based on Ψ_{stem}. A clear pattern was evident in 2018, where most of the southwestern section showed more negative Ψ_{stem} values than the rest of the vineyard (**Figure 4A**). In 2019, a larger area in the central section of the vineyard had more negative Ψ_{stem} values in the plants (**Figure 4C**). Comparing the clustering of both years, there was a 73.2% similarity between the two clusterings in 2018 and 2019 (**Figures 4B,D**).

In 2018, Ψ_{stem} were consistently separated between the two fHZs (**Figure 5A**). The overall Ψ_{stem} values were consistent in 2018. However, there were exceptions to this trend on 20 September and 28 September due to the precipitation took place on 10 September and 23 September. The research site received 0.2 mm of precipitation during this period of time, causing Ψ_{stem} values to increase. In 2019, as the soil gradually dried, the Ψ_{stem} eventually became more negative throughout the

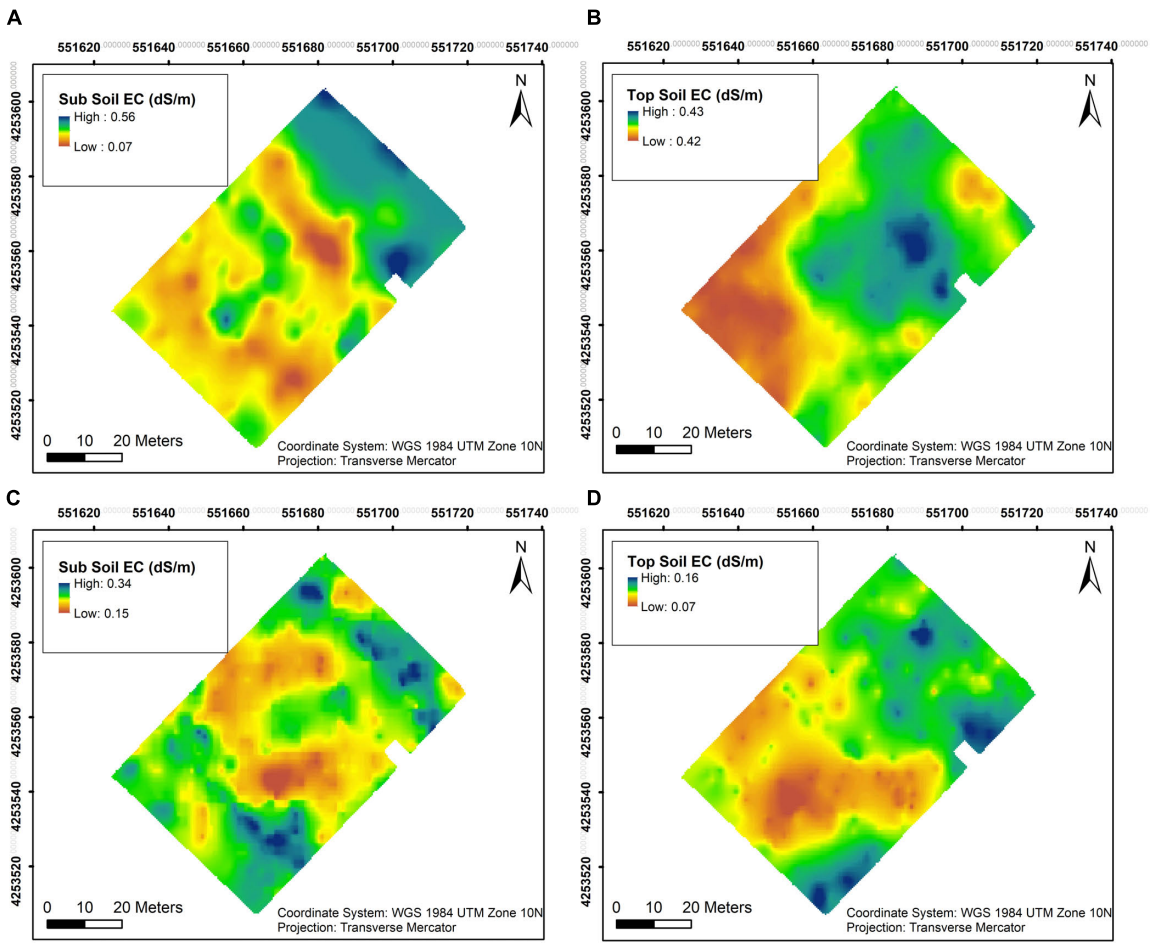

FIGURE 3 | Interpolation maps of soil electrical conductivity (EC) in two depths assessed by EM38 in 2018 and 2019. **(A)** Sub soil EC in 2018, **(B)** top soil EC in 2018, **(C)** sub soil EC in 2019, and **(D)** top soil EC in 2019.

season (**Figure 5B**). Between the two water status zones, Ψ_{stem} were also consistently separated in 2019, and a 0.27 MPa Ψ_{stem} difference was observed between these two fHZs in 2018, but a 0.18 MPa in 2019.

A_n was measured in both years, and the separation between the two water status zones was not evident (**Figures 6A1,A2**). In 2018, the differences in Ψ_{stem} transiently translated into A_n between the two water status zones. We saw differences on 16 August 2018, 15 August 2019, and 29 August 2019, where the moderately water stressed zone had significantly greater A_n. There was a drop in A_n and g_s values on 15 August 2019 due to an extreme weather condition the plants were experiencing with an ambient air temperature of 40.89 ± 0.50°C and a leaf temperature of 45.02 ± 1.48°C. However, this extreme condition did not affect the separations in gas exchange between the two fHZs except it showed an opposite result in WUEi.

The moderately water stressed zone had greater g_s when compared to the severely water stressed zone from 16 August to 2 September in 2018, but there was not difference on the other dates of that season (**Figure 6B1**). In 2019, the same differences between the two fHZs were observed only on 1 August and 15 August with moderately water stressed zone having higher g_s values (**Figure 6B2**).

In contrast to the g_s, WUEi was greater in the severely water stressed zone within the same period of time in 2018 (**Figure 6C1**). In 2019, WUEi was significantly higher in the severely water stressed zone on 1 August and 29 August (**Figure 6C2**). On 15 August 2019, the moderately water stressed zone transiently had higher WUEi than the severely water stressed zone.

Yield Components, Berry Composition, and Berry Skin Flavonoids

Yield components and berry primary metabolites were measured in both 2018 and 2019. In 2018, there was no difference observed in cluster number per vine, cluster weight, berry number per vine, or yield per vine between the two fHZs (**Table 1**). However, berry weight and berry skin weight were greater in the moderately water stressed zone compared to severely water stressed zone. There was no difference in leaf

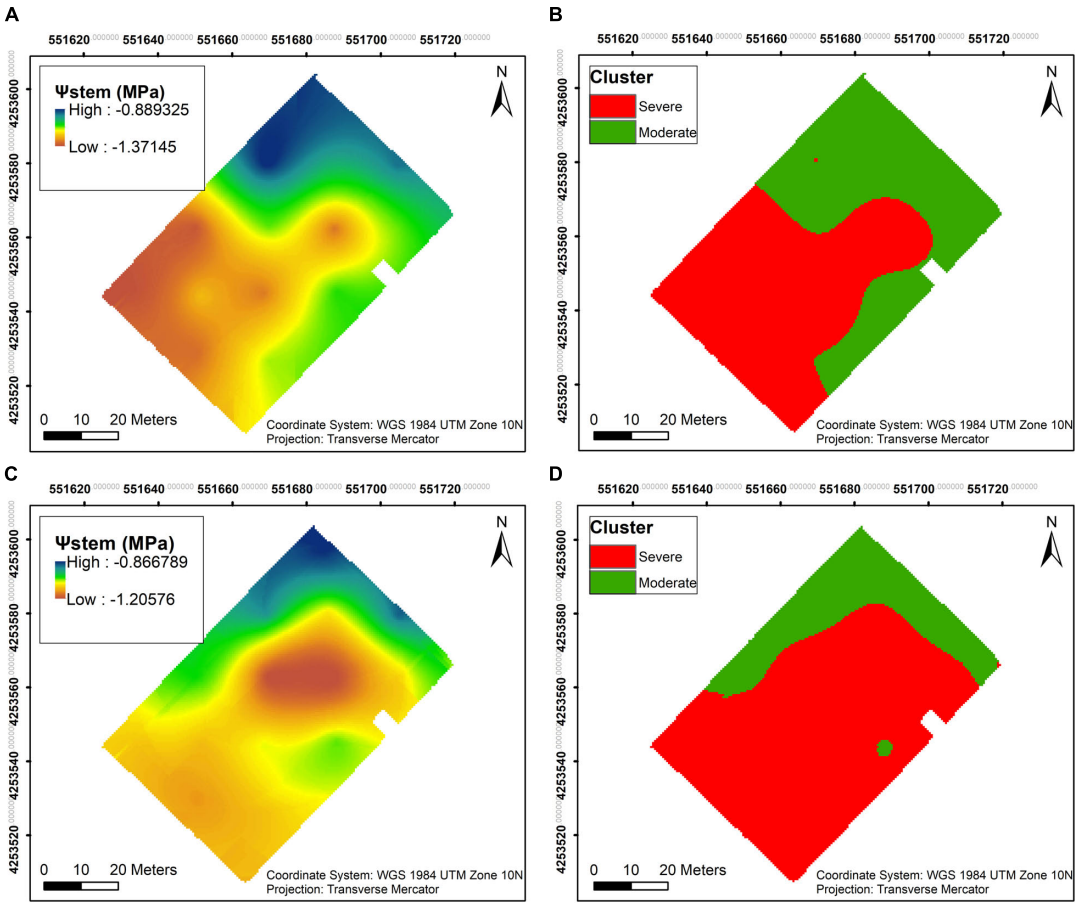

FIGURE 4 | Interpolation maps of plant water status, presented as stem water potential (Ψ_{stem}), and *k*- means clustering maps, delineating the vineyard into two functional homogeneous zones (fHZs) in 2018 and 2019. **(A)** Ψ_{stem} kriging map in 2018, **(B)** *k*- means clustering of Ψ_{stem} integrals in 2018, **(C)** Ψ_{stem} kriging map in 2019, **(D)** *k*- means clustering of Ψ_{stem} integrals in 2019.

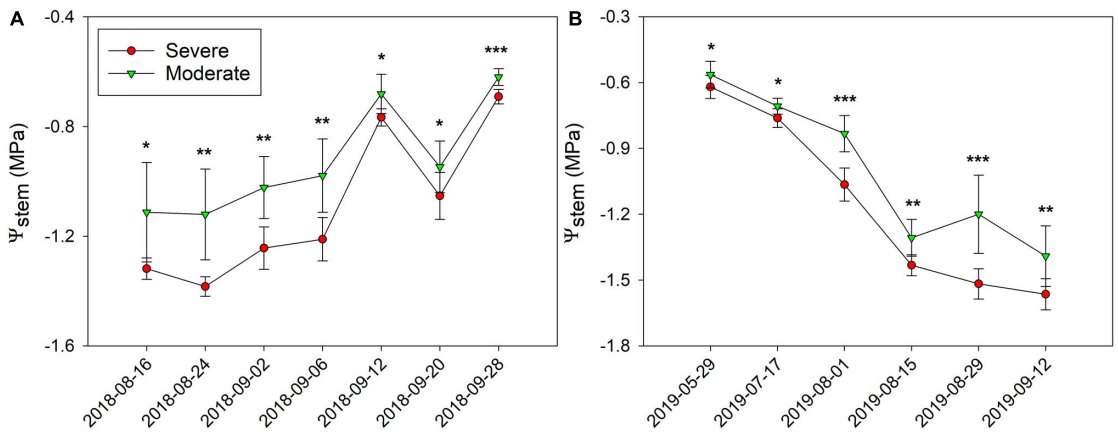

FIGURE 5 | Progression of stem water potential (Ψ_{stem}) between the two functional homogeneous zones (fHZs) in 2018 and 2019. Error bars represent standard deviation from the mean.

area, leaf area to fruit ratio between the two fHZs. The two fHZs had the same berry juice TSS, TA, and pH at harvest in 2018.

In 2019, there was no difference in any of the yield components either (**Table 1**). The fruits showed a more advanced maturity in 2019 compared to the first season. However, there was

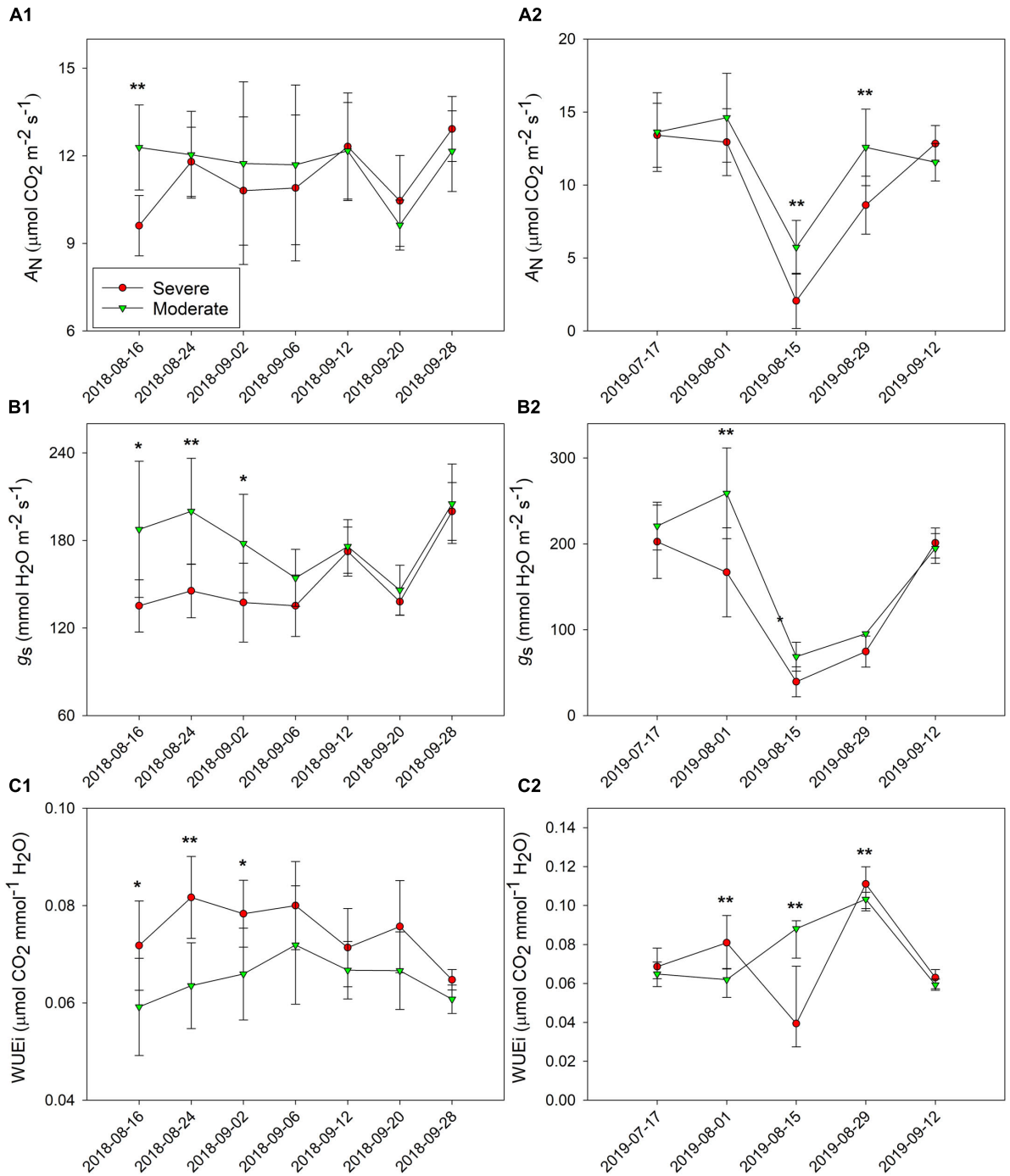

FIGURE 6 | Progression of leaf gas exchanges between the two functional homogeneous zones (fHZs) in 2018 and 2019. **(A)** net carbon assimilation, A_N, **(B)** stomatal conductance, g_s, **(C)** intrinsic water use efficiency, WUE_i, (1) 2018, (2) 2019. Error bars represent standard deviation from the mean.

no difference observed in berry primary metabolites between the two water status zones except TA. The moderately water stressed zone had higher TA than the severely water stressed zone.

Skin flavonols were not generally affected by the spatial variations of plant water status. However, there were differences observed between the two water status zones in the total quercetin and total flavonols on a per berry basis, where the severely water stressed zone had higher quercetin and total flavonols in 2019 (**Table 2**). There was no difference observed in any other flavonol derivatives.

For skin anthocyanins, there was no difference observed in total delphinidin, cyanidin, or petunidin on neither per berry

TABLE 1 | Yield components and berry primary metabolites at harvest of Cabernet Sauvignon as separated by plant water status zoning in Oakville, CA in 2018 and 2019[a].

		Cluster no. per vine	Cluster weight (g)	Yield per vine (kg)	Berry weight (g)	Skin weight (g)	Berry no. per vine	Leaf area (m^2)	Leaf area/ fruit (m^2/kg)	TSS (°Brix)	TA (g L^{-1})	pH
2018	Severe Water Stress ± SD	110.22 ± 19.32	80.03 ± 16.69	8.45 ± 1.08	1.14 ± 0.07 **b**	0.05 ± 0.00 **b**	7387.6 ± 894.48	4.51 ± 1.09	0.55 ± 0.19	21.63 ± 1.22	9.43 ± 0.56	3.24 ± 0.03
	Moderate Water Stress ± SD	98.57 ± 26.78	90.71 ± 9.88	8.82 ± 2.15	1.29 ± 0.05 **a**	0.06 ± 0.01 **a**	6930.9 ± 1783.45	4.33 ± 0.59	0.51 ± 0.10	22.33 ± 1.66	9.45 ± 0.35	3.23 ± 0.04
	p-value	ns	ns	Ns	0.001	0.014	ns	ns	ns	ns	ns	ns
2019	Severe Water Stress ± SD	78.19 ± 17.02	61.35 ± 9.04	4.77 ± 1.18	0.98 ± 0.09	0.07 ± 0.01	5058.34 ± 1304.35	5.72 ± 0.93	1.26 ± 0.36	26.12 ± 1.28	8.29 ± 0.25 **b**	3.41 ± 0.13
	Moderate Water Stress ± SD	89.53 ± 21.95	67.01 ± 5.45	5.96 ± 1.31	1.03 ± 0.06	0.07 ± 0.01	5825.56 ± 1549.39	5.86 ± 0.62	1.01 ± 0.14	27.01 ± 1.23	8.92 ± 0.73 **a**	3.47 ± 0.15
	p value	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.034	ns
Year		0.01571	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	ns	<0.0001	<0.0001	<0.0001	<0.0001
Year × Zoning		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

[a]*ANOVA to compare data (p-value indicated); Letters within columns indicate significant mean separation according to Tukey's HSD test.*

TABLE 2 | Grape berry skin flavonols and anthocyanins at harvest of a Cabernet Sauvignon vineyard as separated by plant water status zoning in Oakville, CA in 2018 and 2019[a].

		Myricetin		Quercetin		Kaempferol		Total flavonols		Delphinidin		Cyanidin		Petunidin		Peonidin		Malvidin		Total anthocyanins	
		mg g^{-1} skin dry wt	mg $berry^{-1}$	mg g^{-1} skin dry wt	mg $berry^{-1}$	mg g^{-1} skin dry wt	mg $berry^{-1}$	mg g^{-1} skin dry wt	mg $berry^{-1}$	mg g^{-1} skin dry wt	mg $berry^{-1}$	mg g^{-1} skin dry wt	mg $berry^{-1}$	mg g^{-1} skin dry wt	mg $berry^{-1}$	mg g^{-1} skin dry wt	mg $berry^{-1}$	mg g^{-1} skin dry wt	mg $berry^{-1}$	mg g^{-1} skin dry wt	mg $berry^{-1}$
2018	Severe Water Stress ± SD	0.87 ± 0.11	0.05 ± 0.00	1.05 ± 0.19	0.06 ± 0.01	0.28 ± 0.05	0.02 ± 0.00	2.78 ± 0.38	0.15 ± 0.01	8.62 ± 0.68	0.39 ± 0.05	1.06 ± 0.07	0.02 ± 0.00	6.13 ± 0.42	0.25 ± 0.03	2.89 ± 0.17	0.10 ± 0.01 **b**	40.07 ± 2.20 **a**	1.33 ± 0.11 **b**	58.77 ± 2.97 **a**	3.22 ± 0.24
	Moderate Water Stress ± SD	0.77 ± 0.13	0.05 ± 0.01	1.00 ± 0.33	0.06 ± 0.02	0.27 ± 0.05	0.02 ± 0.00	2.56 ± 0.52	0.16 ± 0.03	8.02 ± 1.61	0.43 ± 0.12	1.06 ± 0.19	0.03 ± 0.02	5.62 ± 0.83	0.27 ± 0.06	3.04 ± 0.45	0.13 ± 0.03 **a**	36.36 ± 3.32 **b**	1.44 ± 0.09 **a**	54.08 ± 3.72 **b**	3.44 ± 0.33
	p-value	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.078	0.036	0.058	0.026	ns
2019	Severe Water Stress ± SD	0.92 ± 0.16	0.06 ± 0.01	0.58 ± 0.20	0.04 ± 0.01 **b**	0.18 ± 0.03	0.01 ± 0.00	2.07 ± 0.37	0.14 ± 0.02 **b**	3.98 ± 0.53	0.50 ± 0.12	0.38 ± 0.07	0.06 ± 0.01	3.13 ± 0.37	0.35 ± 0.07	2.15 ± 0.20	0.17 ± 0.04	24.06 ± 2.89	2.24 ± 0.14	33.71 ± 3.67	3.33 ± 0.36
	Moderate Water Stress ± SD	1.07 ± 0.26	0.07 ± 0.01	0.76 ± 0.22	0.05 ± 0.01 **a**	0.20 ± 0.04	0.01 ± 0.00	2.42 ± 0.55	0.16 ± 0.03 **a**	4.52 ± 1.01	0.48 ± 0.11	0.45 ± 0.12	0.06 ± 0.02	3.48 ± 0.71	0.34 ± 0.06	2.43 ± 0.41	0.19 ± 0.04	25.55 ± 4.33	2.29 ± 0.08	36.43 ± 6.37	3.37 ± 0.20
	p-value	ns	ns	ns	0.074	ns	ns	ns	0.081	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Year		<0.0001	ns	<0.0001	<0.0001	0.003	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.034	<0.0001	<0.0001	0.001071	ns	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Year × Zoning		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

[a]*ANOVA to compare data (p-value indicated); letters within columns indicate significant mean separation according to Tukey's test.*

basis nor per mg dry skin matter basis in either year (**Table 2**). There was lower total peonidin content per berry in the severely water stressed zone. The most abundant anthocyanin derivative malvidin had a higher concentration in skin dry matter in the severely water stressed zone in 2018. However, this difference in malvidin was reversed when compared on a content per berry basis, where malvidin concentration was lower in skin dry matter in the severely water stressed zone. There was no significant differences observed in any of the anthocyanin derivatives in the second season.

In 2018, there was no difference in di-, tri- hydroxylated flavonols (**Figure 7A**). There was higher proportion of tri-hydroxylated anthocyanins in the severely water stressed zone compared to the other zone, but di-hydroxylated anthocyanin proportion was lower. In 2019, there were no differences between the two water status zones in either flavonol or anthocyanin hydroxylated forms (**Figure 7B**).

Relationship Between Proximal Soil Sensing and Physiological Indicators

The relationships between Ψ_{stem} and soil EC were investigated in both years. Soil EC values increased when the plant water status was more positive (**Figure 8**). In 2018, there was a direct and positive relationship between sub soil EC and Ψ_{stem} integrals (**Figure 8A1**, r^2 = 0.5552, p = 0.0035). A similar relationship was evident between top soil EC and Ψ_{stem} integrals in 2018, albeit not as strong as sub soil EC (**Figure 8A2**, r^2 = 0.2913, p = 0.0569). In 2019, sub soil EC had a moderate linear correlation with Ψ_{stem} (**Figure 8B1**, r^2 = 0.2199, p = 0.1241). There was only a weak linear correlation between top soil EC with Ψ_{stem} (**Figure 8B2**, r^2 = 0.1071, p = 0.2751). These two correlations were not statistically significant in 2019.

The relationships between g_s integrals and soil EC was also investigated. The soil EC values would increase when higher stomatal conductance was measured, but one exception was observed with top soil EC in 2019 (**Figure 9**). In 2018, g_s integrals and sub soil EC were directly and positively related (**Figure 9A1**, r^2 = 0.3895, p = 0.0226). Although the top soil EC and g_s integrals showed a similar trend, they were not directly related (**Figure 9A2**, r^2 = 0.0920, p = 0.3139). In 2019, sub soil EC displayed a similar trend with g_s integrals, however, the relationship between them was not significant (**Figure 9B1**, r^2 = 0.0976, p = 0.3229). The top soil EC was not related to g_s integrals in 2019 (**Figure 9B2**, r^2 = 0.1093, p = 0.2482).

DISCUSSION

Soil Characteristics, Soil EC and Plant Water Status Relationships in Space

Soil texture can play a critical role in determining soil water holding capacity, total transpirable soil water, and plant water status (Pellegrino et al., 2005; Tramontini et al., 2013). The severely water stressed zone had higher a proportion of silt and clay (**Table 3**), but a lower proportion of sand and gravel at the depths of 0.75–1.5 m. It had been shown that sandy soil could contribute to more accessibility of soil water content to the plants than clay soil when only soil texture was considered in the scenario (Tramontini et al., 2013). We attributed this factor to one of the possible reasons why one water status zone was directed toward greater water stress in the plants than the other. However, the same study showed that having more gravels in soil would impose more water stressed conditions with more negative plant water potential and lower stomatal conductance. Our results contradicted this condition where the severely water stressed zone had less gravel proportion than the other zone. We attributed this to the finding that the proportion of gravel between the two fHZs not being different enough to allow this factor to affect water availability.

Previous studies postulated that installing pressurized irrigation systems may ameliorate the natural spatial variability originating from the soil (Chaves et al., 2010; Rogiers et al., 2011). In our work, irrigation was scheduled and applied uniformly throughout the whole growing season in both years. Still, the plant water status was consistently separated between the two water status zones in both years. This aligned with some conclusions made from our previous work, and further corroborated that with uniform irrigation regimes, the plant water status within one vineyard would not necessarily be uniform (Verdugo-Vásquez et al., 2016; Brillante et al., 2017). Especially with extreme weather conditions being prevalent such as heat waves or more than three times of the normal precipitation amount falling on this vineyard, the spatial variability of the soil would still have a dominant effect on plant development and inevitably reveal the pre-existing various characteristics from the soil.

There were many factors that may alter water availability toward plants. Soil electrical properties can reflect many soil characteristics, including soil texture, soil water content, and soil salinity (Bushman and Mehalick, 1989; Bittelli, 2011; Brillante et al., 2015). This approach had already been applied for soil water content and salinity assessment (Brevik et al., 2006; Brunet et al., 2010; Peralta and Costa, 2013; Brillante et al., 2014). In previous studies, soil electrical properties combined with machine-learning algorithms, was utilized as a useful tool to assess plant available soil water (Brillante et al., 2015, 2016c). Also, it can be used as a baseline to immediately identify the variability in vineyard soils, which can direct soil survey with more focused sampling strategies (Bonfante et al., 2015). In our study, soil EC was assessed on the dates close to harvest to validate the possibility of a simple and direct correlations between soil EC and season long plant water status. Soil EC had a moderate to strong correlation with long-term plant water status Ψ_{stem} integrals in both sub and top soil in the first season. The same trend in these relationships were also observed in the second season even though the correlations were not statistically significant. Sub soil EC also showed a significant correlation with g_s integrals in the first year, which had been closely associated with plant water status in previous studies (Costa et al., 2012; Brillante et al., 2017). According to previous research, different varieties responded to water stress differently in terms of controlling stomatal conductance (Rogiers et al., 2011; Pou et al., 2012). Many

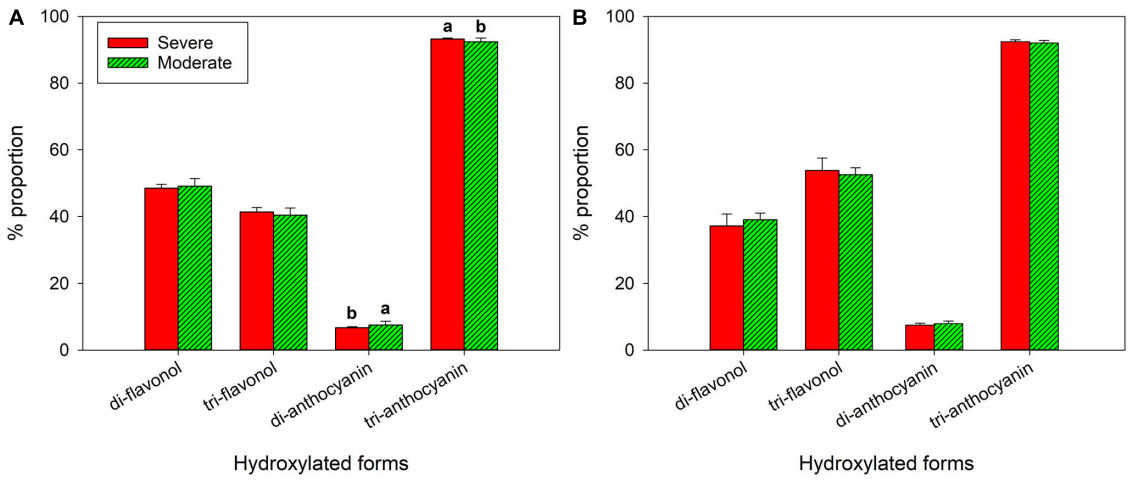

FIGURE 7 | Hydroxylation of flavonols and anthocyanins at harvest of a Cabernet Sauvignon vineyard as separated by plant water status zoning in Oakville, CA in **(A)** 2018 and **(B)** 2019.

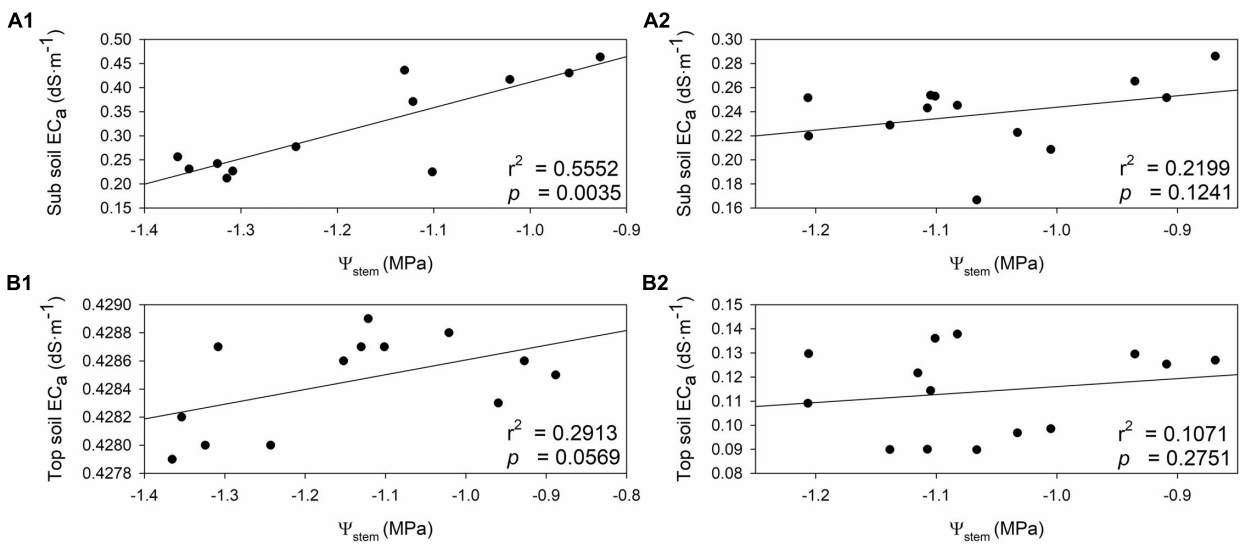

FIGURE 8 | Relationship between stem water potential integrals (Ψ_{stem}) and soil electrical conductivity (EC_a) in two soil depths assessed by EM38 in 2018 and 2019. **(A)** 2018, **(B)** 2019, (1) sub soil EC_a (0.75 – 1.50 m), (2) top soil EC_a (0 – 0.75 m).

cultivars did not respond to plant water status as instantly as some others did since stomatal closure would be maintained by accumulated abscisic acid (ABA) under drought (Tombesi et al., 2015). Top soil EC showed a negative relationship with g_s integrals, which did not correspond to the relationship between the two parameters in 2018, nor reflect the plant water status by Ψ_{stem} integrals observed in 2019. Previous research had suggested that ground-truthing the soil samples were necessary to interpret the soil EC assessment (Morari et al., 2009). Nevertheless, vineyard delineation based on soil electrical properties were still useful to identify the variability in the soil, and plant physiological and chemical properties derived from it (Bramley et al., 2011a; Tagarakis et al., 2013). These results provided the evidence that proximal sensing soil EC could be a plausible and manageable way to assess spatial variation of plant water status.

Due to the natural spatial variability within vineyards, the noticeable spatial non-uniformity in plant water status was reported previously (Brillante et al., 2016b, 2017). The spatial variability in plant water status stemmed from the highly variable soil characteristics within the vineyard according to previous studies (Grote et al., 2010; Taylor et al., 2010; Brillante et al., 2016b). In our previous work we reported that the spatial variability in plant water status altered the leaf gas exchange (Brillante et al., 2017). A more positive plant water status would increase the leaf stomatal conductance and also increase the net carbon assimilation until the plant reaches its photosynthetic capacity

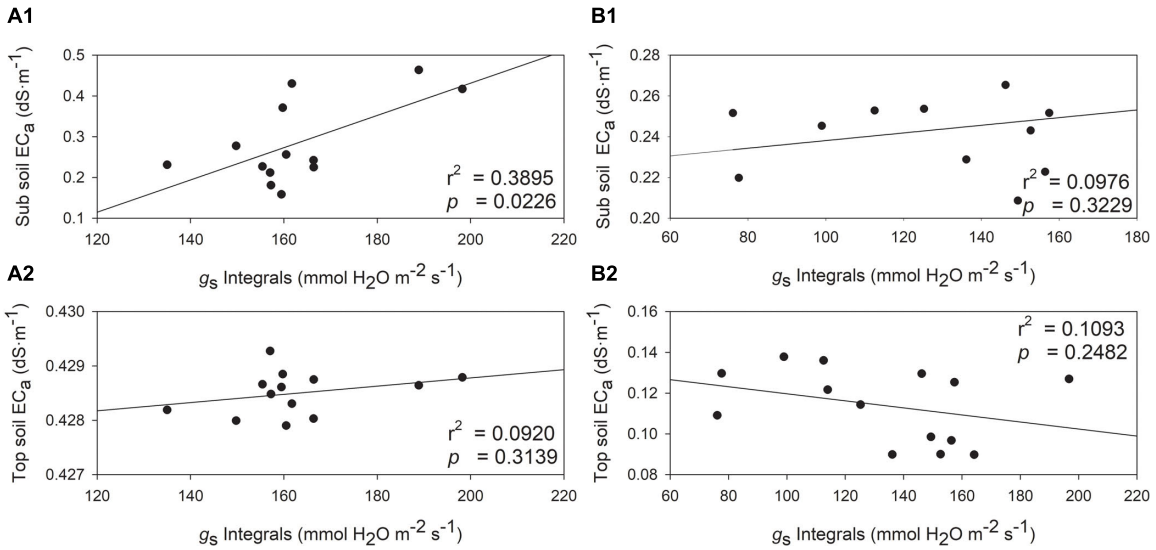

FIGURE 9 | Relationships between stomatal conductance integrals (g_s) and soil electrical conductivity (EC_a) in two depths assessed by EM38 in 2018 and 2019. **(A)** 2018, **(B)** 2019, (1) sub soil EC_a (0.75 – 1.50 m), (2) top soil EC_a (0 – 0.75 m).

TABLE 3 | Soil characteristics assessed at field capacity in 2019 of Cabernet Sauvignon as separated by plant water status zoning in Oakville, CA[a].

		OM	Sand (%)	Silt (%)	Clay (%)	Gravel (%)
Sub soil (0.75 – 1.50 m)	Severe Water Stress ± SD	1.14 ± 0.07	29.00 ± 7.95 **b**	33.67 ± 3.20 **a**	37.33 ± 6.15 **a**	10.32 ± 3.60 **b**
	Moderate Water Stress ± SD	1.14 ± 0.19	41.63 ± 12.25 **a**	28.25 ± 4.65 **b**	30.13 ± 7.86 **b**	14.12 ± 5.79 **a**
	p value	ns	0.026	0.031	0.063	0.086
Top soil (0 – 0.75 m)	Severe Water Stress ± SD	1.93 ± 0.27	41.83 ± 2.48	31.17 ± 1.83	27.00 ± 1.67	13.52 ± 2.66
	Moderate Water Stress ± SD	1.93 ± 0.31	40.00 ± 9.56	29.25 ± 4.03	30.75 ± 7.03	14.84 ± 3.67
	p-value	ns	ns	ns	ns	ns

[a]*ANOVA to compare data (p-value indicated); Letters within columns indicate significant mean separation according to Tukey's HSD test.*

(Flexas et al., 2010; Costa et al., 2012). Although we measured and report these within the confines of study, they were not consistently evident in both years. We attributed this lack of consistency to the highly variable precipitations received at the research vineyard.

Yield Components

Plant water status had been reported to be one of the main factors affecting grapevine yield components in previous studies, where higher water stress could decrease berry weight, berry skin weight, and yield per vine (Castellarin et al., 2007a; Santesteban et al., 2011; Bonada et al., 2013). In our study, we measured less berry weight and berry skin weight in the severely water stressed zone in 2018, but not in 2019. However, the plant water status between the two fHZs were consistently separated in both years. Thus, the inconsistency might be due to the smaller difference in Ψ_{stem} integrals between the two fHZs in 2019, where 0.18 MPa was observed from the start of the season to harvest and 0.16 MPa from veraison to harvest (data not shown) compared to 0.27 MPa in 2018. This was also attributed to the great variation in precipitation received in both years as indicated by the significant Year effect presented herein. We observed no difference in yield per vine between two water status zones, which was also observed previously (Acevedo-Opazo et al., 2010; Brillante et al., 2017). A lacking of water stress severity, and the plant water statuses between the two fHZs were not significantly dissimilar could be the reason that no detrimental yield loss was observed in neither years.

In small plot trials water stress was effective in altering plant canopy development, and leaf area was directly related to canopy microclimate (Intrigliolo and Castel, 2010; Keller et al., 2016; Kraus et al., 2018). Previous studies had also shown that canopy microclimate had a determining role in altering berry chemistry biosynthesis (Cook et al., 2015; Yu et al., 2016). In our current work, we did not observe differences in leaf area even though the plant water status was consistently separated throughout the two seasons. Leaf area to fruit ratio was used to characterize the source-sink relationship (Kliewer and Dokoozlian, 2005; Zufferey et al., 2012). Previous research suggested that to reach the maximum level of maturity, a leaf area to fruit ratio between 0.8 to 1.2 m^2/kg was required for a single-canopy trellis system (Kliewer and Dokoozlian, 2005). In our study, there was no difference of leaf area to fruit ration between two water status zones and the first year had overall leaf to fruit ratio lower than 0.8 m^2/kg consistent with previous findings that mechanically managed

vineyards in warm regions may ripen fruit to technological maturity at lower values (Kurtural et al., 2019).

Berry Primary Metabolisms

The severity of water stress in grapevine is a determining factor in directing berry primary metabolism according to previous studies (Basile et al., 2011; Santesteban et al., 2011). Moderate water stress could lead to a more advanced maturity, which would result in higher TSS and lower TA (Brillante et al., 2017). However, severe water stress may cause a delay in berry development (Korkutal et al., 2011; Martínez-Lüscher et al., 2015). There was no difference observed in any of the berry primary metabolites in either year, except TA in 2019. The difference in plant water status was not greatly different enough to solicit a difference in the berry maturity levels between the two fHZs. Likewise, previous research indicated that A_n was directly related to final TSS accumulation, where higher A_n led to higher TSS (Brillante et al., 2016a). However, our study did not give consistently evident separations in net carbon assimilation. In previous studies, TA reduction after veraison was usually used as an indicator of berry maturity aside from TSS accumulation (Dai et al., 2011). Even though both seasons did not have water stress great enough to alter the TSS accumulation, there was a greater advancement in berry maturity in 2019 compared to 2018. We attributed this to the greater GDD accumulation during 2019 as well as the greater amount of precipitation received. Furthermore, in the first year, leaf area to fruit ratio was lower than the lower limit of the 0.8 m^2/kg requirement. This 'over-cropping' condition might have contributed to the lower TSS in the first year, causing the fruits had a less advanced development to reach the maximum level of maturity. Therefore, the difference observed in TA could be because the malate metabolism became more sensitive toward water stress at a more advanced ripening stage in 2019 (Cramer et al., 2007; Sweetman et al., 2014).

Berry Secondary Metabolisms – Skin Flavonoids

Water stress had been reported to have direct effects on both flavonoid biosynthesis, and flavonoid concentration due to berry dehydration (Castellarin et al., 2007a; Bondada and Shutthanandan, 2012; Brillante et al., 2017). Previous studies had shown that moderate water stress can enhance berry skin flavonoid accumulation as well as the concentration (Bucchetti et al., 2011; Martínez-Lüscher et al., 2014a). However, when the severity of water stress increased even further, the degradation of flavonoid compounds could be more pronounced (Brillante et al., 2017). According to previous research, anthocyanins were more sensitive toward water stress than flavonols (Castellarin et al., 2007a). It was also shown that flavonol accumulation could also be altered by different water deficit irrigation regimes (Zarrouk et al., 2012). However, some studies had pointed out that flavonols were more dominated by solar radiation than water stress, and they were particularly sensitive toward UV-B (Martínez-Lüscher et al., 2014a,b). In our study, higher quercetin and total flavonol content per berry were observed in the moderately water stressed zone. Although the leaf area comparisons within these two fHZs did not reveal a significant difference, we attribute the difference in flavonol profiles to spatial variability of soil water supply as corroborated in our recent work (Martínez-Lüscher et al., 2014b).

The increasing content of total skin anthocyanins with water stress in previous studies agreed with our results in 2018, where the fruits from the severely water stressed zone showed a higher total anthocyanin concentration in the skin tissues (Brillante et al., 2017). This effect was not observed on the content per berry basis. When compared between the two water status zones, moderate water stress led to higher peonidin, malvidin content per berry, yet severe water stress lead to higher malvidin and total skin anthocyanin concentration in skin dry matter. This discrepancy in the weight basis and per berry basis was observed in previous studies (Ginestar et al., 1998; Kennedy et al., 2002). It could be due to the enhanced anthocyanin concentration relative to the skin tissue masses, but less total amount of flavonoid compounds accumulated in each berry in 2018. We could not rule out that the severe water stress may had led toward a greater anthocyanins degradation, or the overall berry development was slightly slowed down like our yield component results presented, causing less flavonoids accumulated. Additionally, the second year showed an overall lower anthocyanin per mg dry skin basis compared to the first year. Previous research showed that an advanced maturity would initiate anthocyanin degradation (Brillante et al., 2017). After around 23°Brix, anthocyanin degradation (on both per g of berry mass basis and per berry basis) would be exacerbated (Martínez-Lüscher et al., 2017, 2019). However, the degradation might not be the only reason to thoroughly explain the phenomenon between these two years since anthocyanin content per berry was higher in 2019 compared to 2018. We observed greater berry skin weight but lower berry weight in 2019 than 2018. Thus, one possibility was that the effect of the advanced berry development on berry physical characteristics overrode the effect on berry skin chemical characteristics. The total anthocyanin content of the whole plant might be lower due to the degradation in 2019 than 2018, but the berry numbers were also lower as observed. The average anthocyanin content per berry can still be higher in 2019 than 2018.

As corroborated by previous studies, the severely water stressed zone had higher proportion of tri-hydroxylated and lower di-hydroxylated anthocyanins when compared to moderately water stressed zone (Castellarin et al., 2007b; Martínez-Lüscher et al., 2014a; Brillante et al., 2017). Previous work provided evidence that, F3′5′H can be upregulated in the flavonoid biosynthetic pathway with moderate water stress (Castellarin et al., 2007a), which would result in increasing hydroxylation level on the B-ring of flavonoid skeleton. Additionally, tri-hydroxylated anthocyanins were more stable against oxidation or degradation than di-hydroxylated forms (Mori et al., 2007), which could be another reason besides F3′5′H upregulation to have a higher proportion with severe water stress. Although the possibly affected transcription factors F3′H and F3′5′H were shared to produce both flavonols and anthocyanins in the same pathway branches, tri- or di-hydroxylated flavonols along were not disparate between the two fHZs in our study.

CONCLUSION

Recent precision viticulture studies had proposed that vineyard delineation can be a plausible approach to monitor and manage spatial variability present in the vineyard (Peralta and Costa, 2013; Tagarakis et al., 2013; González-Fernández et al., 2017). Being a critical physiological parameter, plant water status was able to successfully capture the spatial variability in the final berry chemistry in previous research (Brillante et al., 2017, 2018), and it was further studied in this specific study. Study presented here in provided evidence that the spatial variability within the vineyard can be apparent in plant physiology and berry chemistry. Moreover, our results provided evidence that proximal sensing of soil EC may be a useful tool to connect soil to plant water status, even further to berry primary and secondary chemistry as observed in recent research (Bonfante et al., 2015; Tardaguila et al., 2018; Priori et al., 2019). This fundamental knowledge can contribute to a greater linkage between available sensing technologies and quality-related chemical analysis in precision viticulture research (Matese et al., 2015). The promptness and efficiency of proximal sensing can be transformed into realistic utilization, which can be significantly beneficial in large-acreage vineyards.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

SK designed the project, acquired the funding. RY collected and analyzed the data, wrote the first version of the manuscript. RY and SK interpreted the data, read and approved the final version of the manuscript.

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REFERENCES

Acevedo-Opazo, C., Ortega-Farias, S., and Fuentes, S. (2010). Effects of grapevine (*Vitis vinifera* L.) water status on water consumption, vegetative growth and grape quality: an irrigation scheduling application to achieve regulated deficit irrigation. *Agric. Water Manag.* 97, 956–964. doi: 10.1016/j.agwat.2010.01.025

Basile, B., Marsal, J., Mata, M., Vallverdú, X., Bellvert, J., and Girona, J. (2011). Phenological sensitivity of Cabernet Sauvignon to water stress: vine physiology and berry composition. *Am. J. Enol. Vitic.* 62, 452–461. doi: 10.5344/ajev.2011.11003

Bittelli, M. (2011). Measuring soil water content: a review. *HortTechnology* 21, 293–300. doi: 10.21273/horttech.21.3.293

Bonada, M., Sadras, V., Moran, M., and Fuentes, S. (2013). Elevated temperature and water stress accelerate mesocarp cell death and shrivelling, and decouple sensory traits in Shiraz berries. *Irrig. Sci.* 31, 1317–1331. doi: 10.1007/s00271-013-0407-z

Bondada, B., and Shutthanandan, J. (2012). Understanding differential responses of grapevine (*Vitis vinifera* L.) leaf and fruit to water stress and recovery following re-watering. *Am. J. Plant Sci.* 3, 1232–1240. doi: 10.4236/ajps.2012.39149

Bonfante, A., Agrillo, A., Albrizio, R., Basile, A., Buonomo, R., De Mascellis, R., et al. (2015). Functional homogeneous zones (fhzs) in viticultural zoning procedure: an Italian case study on Aglianico vine. *Soil* 1, 427–441. doi: 10.5194/soil-1-427-2015

Bonfante, A., Alfieri, S., Albrizio, R., Basile, A., De Mascellis, R., Gambuti, A., et al. (2017). Evaluation of the effects of future climate change on grape quality through a physically based model application: a case study for the Aglianico grapevine in Campania region, Italy. *Agric. Syst.* 152, 100–109. doi: 10.1016/j.agsy.2016.12.009

Bonfante, A., Basile, A., Langella, G., Manna, P., and Terribile, F. (2011). A physically oriented approach to analysis and mapping of terroirs. *Geoderma* 167, 103–117. doi: 10.1016/j.geoderma.2011.08.004

Bramley, R. (2005). Understanding variability in winegrape production systems 2. Within vineyard variation in quality over several vintages. *Aust. J. Grape Wine Res.* 11, 33–42. doi: 10.1111/j.1755-0238.2005.tb00277.x

Bramley, R., Ouzman, J., and Boss, P. (2011b). Variation in vine vigour, grape yield and vineyard soils and topography as indicators of variation in the chemical composition of grapes, wine and wine sensory attributes. *Aust. J. Grape Wine Res.* 17, 217–229. doi: 10.1111/j.1755-0238.2011.00136.x

Bramley, R., Ouzman, J., and Thornton, C. (2011a). Selective harvesting is a feasible and profitable strategy even when grape and wine production is geared towards large fermentation volumes. *Aust. J. Grape Wine Res.* 17, 298–305. doi: 10.1111/j.1755-0238.2011.00151.x

Brevik, E. C., Fenton, T. E., and Lazari, A. (2006). Soil electrical conductivity as a function of soil water content and implications for soil mapping. *Precis. Agric.* 7, 393–404. doi: 10.1007/s11119-006-9021-x

Brillante, L., Belfiore, N., Gaiotti, F., Lovat, L., Sansone, L., Poni, S., et al. (2016a). Comparing kaolin and pinolene to improve sustainable grapevine production during drought. *PLoS One* 11:e0156631. doi: 10.1371/journal.pone.0156631

Brillante, L., Bois, B., Lévêque, J., and Mathieu, O. (2016b). Variations in soil-water use by grapevine according to plant water status and soil physical-chemical characteristics—A 3D spatio-temporal analysis. *Eur. J. Agron.* 77, 122–135. doi: 10.1016/j.eja.2016.04.004

Brillante, L., Bois, B., Mathieu, O., Bichet, V., Michot, D., and Lévêque, J. (2014). Monitoring soil volume wetness in heterogeneous soils by electrical resistivity. A field-based pedotransfer function. *J. Hydrol.* 516, 56–66. doi: 10.1016/j.jhydrol.2014.01.052

Brillante, L., Bois, B., Mathieu, O., and Lévêque, J. (2016c). Electrical imaging of soil water availability to grapevine: a benchmark experiment of several machine-learning techniques. *Precis. Agric.* 17, 637–658. doi: 10.1007/s11119-016-9441-1

Brillante, L., Martinez-Luscher, J., Yu, R., Plank, C. M., Sanchez, L., Bates, T. L., et al. (2017). Assessing spatial variability of grape skin flavonoids at the vineyard scale based on plant water status mapping. *J. Agric. Food Chem.* 65, 5255–5265. doi: 10.1021/acs.jafc.7b01749

Brillante, L., Mathieu, O., Bois, B., Van Leeuwen, C., and Lévêque, J. (2015). The use of soil electrical resistivity to monitor plant and soil water relationships in vineyards. *Soil* 1, 273–286. doi: 10.5194/soil-1-273-2015

Brillante, L., Mathieu, O., Lévêque, J., Van Leeuwen, C., and Bois, B. (2018). Water status and must composition in grapevine cv. Chardonnay with different soils and topography and a mini meta-analysis of the δ13C/water potentials correlation. *J. Sci. Food Agric.* 98, 691–697. doi: 10.1002/jsfa.8516

Brunet, P., Clément, R., and Bouvier, C. (2010). Monitoring soil water content and deficit using Electrical Resistivity Tomography (Ert)–A case study in the Cevennes area, France. *J. Hydrol.* 380, 146–153. doi: 10.1016/j.jhydrol.2009.10.032

Bucchetti, B., Matthews, M. A., Falginella, L., Peterlunger, E., and Castellarin, S. D. (2011). Effect of water deficit on Merlot grape tannins and anthocyanins across four seasons. *Sci. Hortic.* 128, 297–305. doi: 10.1016/j.scienta.2011.02.003

Bushman, J. B., and Mehalick, T. E. (1989). "Statistical analysis of soil characteristics to predict mean time to corrosion failure of underground metallic structures," in *Effects of Soil Characteristics on Corrosion*, eds V. Chaker, and J. Palmer (West Conshohocken, PA: ASTM International), 107–118.

Castellarin, S. D., Matthews, M. A., Di Gaspero, G., and Gambetta, G. A. (2007a). Water deficits accelerate ripening and induce changes in gene expression regulating flavonoid biosynthesis in grape berries. *Planta* 227, 101–112. doi: 10.1007/s00425-007-0598-8

Castellarin, S. D., Pfeiffer, A., Sivilotti, P., Degan, M., Peterlunger, E., and Di Gaspero, G. (2007b). Transcriptional regulation of anthocyanin biosynthesis in ripening fruits of grapevine under seasonal water deficit. *Plant Cell Environ.* 30, 1381–1399. doi: 10.1111/j.1365-3040.2007.01716.x

Charrad, M., Ghazzali, N., Boiteau, V., and Niknafs, A. (2014). NbClust: an R package for determining the relevant number of clusters in a data set. *J. Stat. Softw.* 61, 1–36.

Chaves, M., Zarrouk, O., Francisco, R., Costa, J., Santos, T., Regalado, A., et al. (2010). Grapevine under deficit irrigation: hints from physiological and molecular data. *Ann. Bot.* 105, 661–676. doi: 10.1093/aob/mcq030

Cook, M. G., Zhang, Y., Nelson, C. J., Gambetta, G., Kennedy, J. A., and Kurtural, S. K. (2015). Anthocyanin composition of Merlot is ameliorated by light microclimate and irrigation in central California. *Am. J. Enol. Vitic.* 66, 266–278. doi: 10.5344/ajev.2015.15006

Costa, J. M., Ortuño, M. F., Lopes, C. M., and Chaves, M. M. (2012). Grapevine varieties exhibiting differences in stomatal response to water deficit. *Funct. Plant Biol.* 39, 179–189.

Costantini, E. A., Pellegrini, S., Bucelli, P., Barbetti, R., Campagnolo, S., Storchi, P., et al. (2010). Mapping suitability for Sangiovese wine by means of δ13C and geophysical sensors in soils with moderate salinity. *Eur. J. Agron.* 33, 208–217. doi: 10.1016/j.eja.2010.05.007

Cramer, G. R., Ergül, A., Grimplet, J., Tillett, R. L., Tattersall, E. A., Bohlman, M. C., et al. (2007). Water and salinity stress in grapevines: early and late changes in transcript and metabolite profiles. *Funct. Integr. Genomics* 7, 111–134. doi: 10.1007/s10142-006-0039-y

Dai, Z. W., Ollat, N., Gomès, E., Decroocq, S., Tandonnet, J.-P., Bordenave, L., et al. (2011). Ecophysiological, genetic, and molecular causes of variation in grape berry weight and composition: a review. *Am. J. Enol. Vitic.* 62, 413–425. doi: 10.5344/ajev.2011.10116

De Bei, R., Fuentes, S., Gilliham, M., Tyerman, S., Edwards, E., Bianchini, N., et al. (2016). VitiCanopy: a free computer App to estimate canopy vigor and porosity for grapevine. *Sensors* 16:E585. doi: 10.3390/s16040585

Escalona, J., Bota, J., and Medrano, H. (2015). Distribution of leaf photosynthesis and transpiration within grapevine canopies under different drought conditions. *Vitis J. Grapevine Res.* 42, 57–64.

Flexas, J., Galmés, J., Gallé, A., Gulías, J., Pou, A., Ribas-Carbo, M., et al. (2010). Improving water use efficiency in grapevines: potential physiological targets for biotechnological improvement. *Aust. J. Grape Wine Res.* 16, 106–121. doi: 10.1111/j.1755-0238.2009.00057.x

Ginestar, C., Eastham, J., Gray, S., and Iland, P. (1998). Use of sap-flow sensors to schedule vineyard irrigation. Ii. Effects of post-veraison water deficits on composition of shiraz grapes. *Am. J. Enol. Vitic.* 49, 421–428.

Gómez-Míguez, M. J., Gómez-Míguez, M., Vicario, I. M., and Heredia, F. J. (2007). Assessment of colour and aroma in white wines vinifications: effects of grape maturity and soil type. *J. Food Eng.* 79, 758–764. doi: 10.1016/j.jfoodeng.2006.02.038

González-Fernández, A. B., Rodríguez-Pérez, J. R., Ablanedo, E. S., and Ordoñez, C. (2017). Vineyard zone delineation by cluster classification based on annual grape and vine characteristics. *Precis. Agric.* 18, 525–573. doi: 10.1007/s11119-016-9475-4

Grote, K., Anger, C., Kelly, B., Hubbard, S., and Rubin, Y. (2010). Characterization of soil water content variability and soil texture using Gpr groundwave techniques. *J. Environ. Eng. Geophys.* 15, 93–110. doi: 10.2113/jeeg15.3.93

Hardie, M., and Doyle, R. (2012). "Measuring soil salinity," in *Plant Salt Tolerance*, Vol. 913, eds S. Shabala, and T. Cuin (Totowa, NJ: Humana Press).

Hiemstra, P. (2013). *Package 'automap' R Package*. Available online at: https://CRAN.R-project.org/package=automap (accessed August 27, 2013).

Intrigliolo, D. S., and Castel, J. R. (2010). Response of grapevine cv.'Tempranillo'to timing and amount of irrigation: water relations, vine growth, yield and berry and wine composition. *Irrig. Sci.* 28, 113–125. doi: 10.1007/s00271-009-0164-1

Keller, M., Romero, P., Gohil, H., Smithyman, R. P., Riley, W. R., Casassa, L. F., et al. (2016). Deficit irrigation alters grapevine growth, physiology, and fruit microclimate. *Am. J. Enol. Vitic.* 67, 426–435. doi: 10.5344/ajev.2016.16032

Kennedy, J. A., Matthews, M. A., and Waterhouse, A. L. (2002). Effect of maturity and vine water status on grape skin and wine flavonoids. *Am. J. Enol. Vitic.* 53, 268–274.

Kliewer, W. M., and Dokoozlian, N. K. (2005). Leaf area/crop weight ratios of grapevines: influence on fruit composition and wine quality. *Am. J. Enol. Vitic.* 56, 170–181.

Kontoudakis, N., Esteruelas, M., Fort, F., Canals, J. M., De Freitas, V., and Zamora, F. (2011). Influence of the heterogeneity of grape phenolic maturity on wine composition and quality. *Food Chem.* 124, 767–774. doi: 10.1016/j.foodchem.2010.06.093

Korkutal, I., Bahar, E., and Carbonneau, A. (2011). Growth and yield responses of cv. Merlot (*Vitis vinifera* L.) to early water stress. *Afr. J. Agric. Res.* 6, 6281–6288.

Kraus, C., Pennington, T., Herzog, K., Hecht, A., Fischer, M., Voegele, R. T., et al. (2018). Effects of canopy architecture and microclimate on grapevine health in two training systems. *Vitis* 57, 53–60.

Kurtural, S. K., Beebe, A. E., Martínez-Lüscher, J., Zhuang, S., Lund, K. T., Mcgourty, G., et al. (2019). Conversion to mechanical pruning in vineyards maintains fruit composition while reducing labor costs in 'Merlot' grape production. *HortTechnology*, 1, 1–12. doi: 10.21273/HORTTECH04204-18

Liu, Y., Tikunov, Y., Schouten, R. E., Marcelis, L. F., Visser, R. G., and Bovy, A. (2018). Anthocyanin biosynthesis and degradation mechanisms in Solanaceous vegetables: a review. *Front. Chem.* 6:52. doi: 10.3389/fchem.2018.00052

Lorrain, B., Ky, I., Pechamat, L., and Teissedre, P.-L. (2013). Evolution of analysis of polyhenols from grapes, wines, and extracts. *Molecules* 18, 1076–1100. doi: 10.3390/molecules18011076

Martínez-Lüscher, J., Chen, C. C. L., Brillante, L., and Kurtural, S. K. (2017). Partial solar radiation exclusion with color shade nets reduces the degradation of organic acids and flavonoids of grape berry (*Vitis vinifera* L.). *J. Agric. Food Chem.* 65, 10693–10702. doi: 10.1021/acs.jafc.7b04163

Martínez-Lüscher, J., Morales, F., Delrot, S., Sánchez-Díaz, M., Gomès, E., Aguirreolea, J., et al. (2015). Characterization of the adaptive response of grapevine (cv. Tempranillo) to Uv-B radiation under water deficit conditions. *Plant Sci.* 232, 13–22. doi: 10.1016/j.plantsci.2014.12.013

Martínez-Lüscher, J., Plank, C. M., Brillante, L., Cooper, M. L., Smith, R. J., Al-Rwahnih, M., et al. (2019). Grapevine red blotch virus may reduce carbon translocation leading to impaired grape berry ripening. *J. Agric. Food Chem.* 67, 2437–2448. doi: 10.1021/acs.jafc.8b05555

Martínez-Lüscher, J., Sánchez-Díaz, M., Delrot, S., Aguirreolea, J., Pascual, I., and Gomès, E. (2014a). Ultraviolet-B radiation and water deficit interact to alter flavonol and anthocyanin profiles in grapevine berries through transcriptomic regulation. *Plant Cell Physiol.* 55, 1925–1936. doi: 10.1093/pcp/pcu121

Martínez-Lüscher, J., Torres, N., Hilbert, G., Richard, T., Sánchez-Díaz, M., Delrot, S., et al. (2014b). Ultraviolet-B radiation modifies the quantitative and qualitative profile of flavonoids and amino acids in grape berries. *Phytochemistry* 102, 106–114. doi: 10.1016/j.phytochem.2014.03.014

Matese, A., Toscano, P., Di Gennaro, S., Genesio, L., Vaccari, F., Primicerio, J., et al. (2015). Intercomparison of Uav, aircraft and satellite remote sensing platforms for precision viticulture. *Remote Sens.* 7, 2971–2990. doi: 10.3390/rs70302971

Morari, F., Castrignanò, A., and Pagliarin, C. (2009). Application of multivariate geostatistics in delineating management zones within a gravelly vineyard using geo-electrical sensors. *Comput. Electron. Agric.* 68, 97–107. doi: 10.1016/j.compag.2009.05.003

Mori, K., Goto-Yamamoto, N., Kitayama, M., and Hashizume, K. (2007). Loss of anthocyanins in red-wine grape under high temperature. *J. Exp. Bot.* 58, 1935–1945. doi: 10.1093/jxb/erm055

Myers, B. J. (1988). Water stress integral—a link between short-term stress and long-term growth. *Tree Physiol.* 4, 315–323. doi: 10.1093/treephys/4.4.315

Parr, W. V., Green, J. A., White, K. G., and Sherlock, R. R. (2007). The distinctive flavour of New Zealand Sauvignon blanc: sensory characterisation by wine professionals. *Food Qual. Prefer.* 18, 849–861. doi: 10.1016/j.foodqual.2007.02.001

Pebesma, E. J. (2004). Multivariable geostatistics in S: the gstat package. *Comput. Geosci.* 30, 683–691. doi: 10.1016/j.cageo.2004.03.012

Pellegrino, A., Lebon, E., Voltz, M., and Wery, J. (2005). Relationships between plant and soil water status in vine (*Vitis vinifera* L.). *Plant Soil* 266, 129–142. doi: 10.1007/s11104-005-0874-y

Peralta, N. R., and Costa, J. L. (2013). Delineation of management zones with soil apparent electrical conductivity to improve nutrient management. *Comput. Electron. Agric.* 99, 218–226. doi: 10.1016/j.compag.2013.09.014

Pou, A., Medrano, H., Tomàs, M., Martorell, S., Ribas-Carbó, M., and Flexas, J. (2012). Anisohydric behaviour in grapevines results in better performance under moderate water stress and recovery than isohydric behaviour. *Plant Soil* 359, 335–349. doi: 10.1007/s11104-012-1206-7

Priori, S., Pellegrini, S., Perria, R., Puccioni, S., Storchi, P., Valboa, G., et al. (2019). Scale effect of terroir under three contrasting vintages in the Chianti Classico area (Tuscany, Italy). *Geoderma* 334, 99–112. doi: 10.1016/j.geoderma.2018.07.048

R Core Team (2019). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.

Rogiers, S. Y., Greer, D. H., Hatfield, J. M., Hutton, R. J., Clarke, S. J., Hutchinson, P. A., et al. (2011). Stomatal response of an anisohydric grapevine cultivar to evaporative demand, available soil moisture and abscisic acid. *Tree Physiol.* 32, 249–261. doi: 10.1093/treephys/tpr131

Santesteban, L., Miranda, C., and Royo, J. (2011). Regulated deficit irrigation effects on growth, yield, grape quality and individual anthocyanin composition in *Vitis vinifera* L. cv.'Tempranillo'. *Agric. Water Manag.* 98, 1171–1179. doi: 10.1016/j.agwat.2011.02.011

Smart, R. E., and Coombe, B. G. (1983). "Water relations of grapevines," in *Water Deficits and Plant Growth*, ed. T. T. Kozlowski (New York, NY: Academic Press), 137–196. doi: 10.1016/b978-0-12-424157-2.50010-0

Su, S. L., Singh, D., and Baghini, M. S. (2014). A critical review of soil moisture measurement. *Measurement* 54, 92–105. doi: 10.1016/j.envpol.2012.02.026

Sweetman, C., Sadras, V., Hancock, R., Soole, K., and Ford, C. (2014). Metabolic effects of elevated temperature on organic acid degradation in ripening *Vitis vinifera* fruit. *J. Exp. Bot.* 65, 5975–5988. doi: 10.1093/jxb/eru343

Tagarakis, A., Liakos, V., Fountas, S., Koundouras, S., and Gemtos, T. (2013). Management zones delineation using fuzzy clustering techniques in grapevines. *Precis. Agric.* 14, 18–39. doi: 10.1007/s11119-012-9275-4

Tardaguila, J., Diago, M. P., Priori, S., and Oliveira, M. (2018). Mapping and managing vineyard homogeneous zones through proximal geoelectrical sensing. *Arch. Agron. Soil Sci.* 64, 409–418. doi: 10.1080/03650340.2017.1359411

Taylor, J. A., Acevedo-Opazo, C., Ojeda, H., and Tisseyre, B. (2010). Identification and significance of sources of spatial variation in grapevine water status. *Aust. J. Grape Wine Res.* 16, 218–226. doi: 10.1111/j.1755-0238.2009.00066.x

Tombesi, S., Nardini, A., Frioni, T., Soccolini, M., Zadra, C., Farinelli, D., et al. (2015). Stomatal closure is induced by hydraulic signals and maintained by ABA in drought-stressed grapevine. *Sci. Rep.* 5, 1–12. doi: 10.1038/srep12449

Tramontini, S., Van Leeuwen, C., Domec, J.-C., Destrac-Irvine, A., Basteau, C., Vitali, M., et al. (2013). Impact of soil texture and water availability on the hydraulic control of plant and grape-berry development. *Plant Soil* 368, 215–230. doi: 10.1007/s11104-012-1507-x

Verdugo-Vásquez, N., Acevedo-Opazo, C., Valdés-Gómez, H., Araya-Alman, M., Ingram, B., De Cortázar-Atauri, I. G., et al. (2016). Spatial variability of phenology in two irrigated grapevine cultivar growing under semi-arid conditions. *Precis. Agric.* 17, 218–245. doi: 10.1007/s11119-015-9418-5

Williams, L., and Ayars, J. (2005). Grapevine water use and the crop coefficient are linear functions of the shaded area measured beneath the canopy. *Agric. For. Meteorol.* 132, 201–211. doi: 10.1016/j.agrformet.2005.07.010

Yu, R., Cook, M. G., Yacco, R. S., Watrelot, A. A., Gambetta, G., Kennedy, J. A., et al. (2016). Effects of leaf removal and applied water on flavonoid accumulation in grapevine (*Vitis vinifera* L. cv. Merlot) Berry in a Hot Climate. *J. Agric. Food Chem.* 64, 8118–8127. doi: 10.1021/acs.jafc.6b03748

Zarrouk, O., Francisco, R., Pinto-Marijuan, M., Brossa, R., Santos, R. R., Pinheiro, C., et al. (2012). Impact of irrigation regime on berry development and flavonoids composition in Aragonez (Syn. Tempranillo) grapevine. *Agric. Water Manag.* 114, 18–29. doi: 10.1016/j.agwat.2012.06.018

Zufferey, V., Murisier, F., Vivin, P., Belcher, S., Lorenzini, F., Spring, J.-L., et al. (2012). Carbohydrate reserves in grapevine (*Vitis vinifera* L.'Chasselas'): the influence of the leaf to fruit ratio. *Vitis* 51, 103–110.

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Temperature Variability at Local Scale in the Bordeaux Area. Relations With Environmental Factors and Impact on Vine Phenology

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Climate is a major factor of the physical environment influencing terroir expression in viticulture. Thermal conditions strongly impact vine development and grape composition. Spatializing this parameter at local scale allows for more refined vineyard management. In this study, temperature variability was investigated over an area of 19,233 ha within the appellations of Saint-Émilion, Pomerol, and their satellites (Bordeaux, France). A network of 90 temperature sensors was deployed inside grapevine canopies of this area and temperatures were measured from 2012 through 2018. To determine the effect of temperature on vine development, the phenological stages (budbreak, flowering, and véraison) were recorded on 60 reference plots planted with *Vitis vinifera* L. cv. Merlot located near the temperature sensors. Results showed great spatial variability in temperature, especially minimum temperature, with an amplitude of up to 10°C on a given day. The spatial variability of the Winkler index measured in the canopy inside a given vintage was around 320 degree-days. This research explores the main factors affecting spatial variability in temperature, such as environmental factors and meteorological conditions. The impact of temperature on vine behavior was also analyzed. Observed phenological dates were compared to those estimated using the Grapevine Flowering Véraison model. Maps of temperatures and phenological observations were created over this area and provided a useful tool for improved adaptation of plant material and training systems to local temperature variability and change.

Keywords: climate, local scale, viticulture, spatial modeling, vine development, phenology, terroir

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INTRODUCTION

Climate is a major factor of the physical environment influencing terroir expression in viticulture (van Leeuwen et al., 2004; Jones, 2018). Climate, and particularly temperature, determine to a large extent the growing areas well adapted for quality viticulture. Such areas are located mainly between the latitudes 30 and 50°N and 30 and 40°S, with average temperature ranging from

12 to 22°C across the growing season (Gladstones, 1992; Jones et al., 2012). Production of high quality wine grapes requires temperatures that allow ripening in a specific period of the year, ideally in September or early October in the northern hemisphere (van Leeuwen and Seguin, 2006). Extreme temperatures are not beneficial for vine development and grape quality. High temperatures (>35°C) can induce leaf or bunch damage, reduce photosynthesis, and decrease anthocyanin concentrations (Kriedemann and Smart, 1971; Spayd et al., 2002; Mori et al., 2007). Extreme negative temperatures (<-15°C) during winter are likely to cause permanent damage to wood and winter buds, possibly leading to vine death. Impact of negative winter temperatures depend on many parameters like genotype, environment, cultural practices, duration of frost exposure, and tissue hydration (Zabadal et al., 2007; Ferguson et al., 2014). Temperatures below −2.2°C after budbreak can damage young shoots and severely reduce production without, however, killing the vines (Poling, 2008; Dami et al., 2012).

Air temperature strongly impacts vine development and the timing of phenological stages (De Cortázar-Atauri et al., 2009; Parker et al., 2011, 2013, 2020; Chuine et al., 2013) and also grape composition (Mira and de Orduña, 2010). Sugar and acidity content at harvest are related to temperature (Coombe, 1987). This is also the case for secondary metabolites like anthocyanins, which increase with increased temperature up to a threshold and then decline (Spayd et al., 2002; Mori et al., 2007; Tarara et al., 2008). Temperature also impacts aromas and flavor precursors like metoxypyrazines (including IBMP; green pepper flavor), which decrease with higher temperature during the growing season (Falcão et al., 2007). Trimethyl dihydronapthalene (TDN; notes of kerosene), massoia lactone (dried figs and coconut flavors), and γ-nonalactone (cooked peaches flavor) concentrations, are higher in wines made from grapes ripened under warmer conditions (Marais et al., 1992; Pons et al., 2017) which is rather a negative effect on wine quality.

Considering that thermal conditions strongly impact vine development and grape composition, characterizing this parameter is highly important. Several temperature indicators were developed to characterize wine production areas. The Winkler and the Huglin indices (Winkler, 1974; Huglin and Schneider, 1998) or the Average Growing Season Temperature (Jones, 2006) are simple indicators based on the growing season air temperature and allow classification of wine producing areas. Depending on the objectives of climate zoning, it may be appropriate to use a multi-criteria approach (Tonietto and Carbonneau, 2004).

Climate varies temporally and spatially and the annual temporal variations impacting vine development and grape quality potential are considered part of the vintage effect (van Leeuwen et al., 2004; Ubalde et al., 2010). The spatial climate variability has an impact on grapevine variety distribution, vine training system, technical management, and wine styles (Gladstones, 2011). Climate can be reduced to several different scales from macroclimate to microclimate and these scales are inter-dependent (Hess, 1974; Neethling et al., 2019). The spatial variability of climate at a local scale can be highly important and in some cases even more so than variability at large scale, due the influence of local parameters like relief, human infrastructure, vegetation, or bodies of water (Quénol, 2014). The high local temperature variability also depends on the different energy transfer processes between the atmosphere and the surface, thus characterizing the energy balance. It is the ratio between energy input and losses that will determine the air temperature. The energy balance is strongly determined by surface characteristics and atmospheric conditions (solar radiation, cloud cover, wind conditions). The spatial variability of temperatures is higher in anticyclonic atmospheric situations (calm and clear skies) than in low pressure situations (cloudy skies and wind). Cloud cover and wind have a homogenizing effect on temperatures, which reduces the impact of surface characteristics (e.g., topography) on the spatial distribution of temperatures (Guyot, 1997). For these reasons, it is of interest to characterize climate at local scale in winegrowing areas.

The study of climate at local scales requires appropriate measurement networks and climate models. Climate has historically been studied at global and regional scales (continent, country, wide region) by using weather station data from national networks or simulated data from climate models. Weather stations only produce point data and the network mesh is not fine enough to study local climates. Over the past few years, many studies of applied climatology have required the installation of measurement networks at local scales and the development of modeling tools adapted to these scales (Joly et al., 2003; Stahl et al., 2006; Bonnefoy et al., 2013; Wu and Li, 2013; Quénol et al., 2017). Different types of models exist to represent climate at various scales. At the global scale, general circulation models (GCMs) are mainly used to build climate change scenarios that estimate trends in climate variables at low spatial resolution. Global climate models (GCMs) have a resolution of several tens to hundreds of kilometers (IPCC, 2013). Obviously, these types of models cannot take into account the influence of local effects related to surface characteristics. Downscaling methods are therefore used to integrate the effects of surface characteristics to increase the spatial resolution of the models (Daniels et al., 2012). Regional climate models (RCMs) are downscaled global climate models that aim to regionalize the outputs of global models by using nesting of model grids of increasing resolution (Rhoades et al., 2015). In viticulture research, regional climate models (RCMs) were used to produce climate maps at regional scales in Marlborough region in New Zealand (Sturman et al., 2017), in Stellenbosch winegrowing area in South Africa (Bonnardot and Cautenet, 2009), and in Burgundi (Xu et al., 2012). Recent technological development, including miniaturization of temperature sensors and shelters, development of weather stations, as well as the use of digital elevation models (DEMs), geographic information systems (GISs), geostatistics, linear, and non-linear regression modeling allow mapping of air temperatures across winegrowing areas at an even finer scale. Temperature variability was characterized at the regional scale by using weather station networks (Madelin and Beltrando, 2005; Bois, 2007; Cuccia, 2013). More recently, temperature variability was characterized at the local scale by using temperature sensor networks

deployed in vineyards (Bonnardot et al., 2012; Bonnefoy, 2013; Le Roux et al., 2017a).

Precise knowledge of temperature distribution at high spatial resolution allows growers to optimize viticultural practices and selection of plant material according to the local conditions. This issue becomes even more strategic in a context of global warming, where growers need to adapt to spatial temperature variability and evolution over time. There is a wide consensus in the scientific community that the climate is changing (IPCC, 2013), and the recent increase of temperature has already affected vine development, advancing in particular the timing of phenological stages (Bock et al., 2011; Tomasi et al., 2011; Duchêne et al., 2012; van Leeuwen et al., 2019), and modifying grape composition resulting in higher levels of potential alcohol and reduced acidity (Duchêne and Schneider, 2005; van Leeuwen et al., 2019) and wine aromas (Pons et al., 2017).

Considering the evolution of climatic conditions and the objective to preserve quality potential and wine typicity, growers will have to adjust viticultural techniques such as leaf area to fruit weight ratio, timing of pruning, or modify rootstocks, cultivars, or clones (van Leeuwen and Destrac-Irvine, 2017).

In this context, temperature variability was investigated over an area of 19,233 ha within the appellations of Saint-Émilion, Pomerol and their satellites (Bordeaux, France) from 2012 through 2018. The objectives of this study are: (i) to analyze daily and seasonal spatial and temporal temperature variability at local scale, (ii) to create maps of temperature and agro-climatic indices through spatial modeling, (iii) to assess environmental factors impacting spatial temperature variability, (iv) to assess the impact of temperature on vine development and grape composition at ripeness by means of a phenological model, and (v) to create maps of the occurrence of phenological stages.

MATERIALS AND METHODS

Study Area

Saint-Émilion, Pomerol, and their satellite appellations are located in the eastern part of the Bordeaux area, on the right bank of the Dordogne River, about 40 km of the town of Bordeaux (**Figure 1**). This area principally produces red wine and the main varieties grown are *Vitis vinifera* L. cv. Merlot (approximately 75%), *V. vinifera* L. cv. Cabernet franc (approximately 16%), and *V. vinifera* L. cv. Cabernet-Sauvignon (approximately 8%) (Cocks et al., 2014). Most vineyards are planted at densities between 5000 and 6000 vines per hectare. Vines are Guyot pruned and the training system is vertical shoot positioning (VSP trellis). For vineyard floor management, cover crop is widely used in particular on hillside vineyards to prevent erosion.

This area is characterized by several large tertiary (Paleogene) limestone plateaus at approximately 100 m in altitude, shaped by the erosion of the rivers which flow south (Dordogne) and north-west (Isle) of the study site. On the valley floors, gravelly and sandy soils have developed on quaternary alluvium (van Leeuwen et al., 1989). Relief was characterized by an altitude between 2 and 107 m, slopes up to 45% and various exposures.

Climate is oceanic and temperate (Köppen and Geiger, 1954). Total yearly rainfall is 788mm $\pm$ 132.9, and the mean annual temperature is 13.9°C $\pm$ 0.5 (Data: Meteo France weather station of Saint-Émilion, average 1995–2018). Rainfall is well distributed all along the year but slightly lower in the summer (**Figure 2**).

Temperature Monitoring

A Dense Network of Temperature Sensors

In order to characterize temperature variability over this area of 19,233 ha, including 12,200 ha of vineyards, a network of 90 temperature sensors was deployed at the end of 2011. This represents a density of one sensor for 214 ha. At this local scale, it is important to take into account the topography (exposure, slope, elevation), the latitude and longitude, and also local parameters, such as rivers and urban areas, which can potentially influence spatial distribution of temperature (**Figure 1**). Temperature sensors and data loggers were distributed to cover as much as possible the variability of these local parameters.

The data loggers used in this project from 2012 to 2015 are Tinytag Talk2-TK-4023 (Gemini Data Loggers, United Kingdom). From 2016 to 2018, automatic data recovery was achieved by using the LoRa technology, with new data loggers developed by OrbiWise company (Geneva, Switzerland). The thermistor probes used throughout the project from 2012 to 2018 were PB-5005-0M6 (Gemini Data Loggers, United Kingdom). The data loggers were set up on vine posts in the vineyard plots in order to measure temperature as close as possible to the vines. The probes were installed inside solar radiation shields (Type RS3), at a height of 1.2 m close to the vegetation. To reduce measurement errors due to vegetation located just around the solar radiation shields, vine shoots were regularly removed during the vegetative period (Madelin et al., 2014).

The data loggers recorded both minimum (Tn) and maximum (Tx) hourly temperatures. For data treatment, daily temperatures are used in this study. The daily minimum temperature corresponds to the extreme minimum temperature between the day before at 6 pm and the day at 7 pm, and the maximum temperature corresponds to the extreme maximum temperature between the day at 6 am and the day after at 7 am. Average daily temperature was computed as $(T_n + T_x)/2$. Theoretical accuracy of the data logger is 0.4°C and to reduce measurement uncertainties, two thermistor probes were installed in every solar shield starting in 2017. This allows detecting deviation and erroneous temperatures due to sensor problems.

From 2012 to 2017, the quality of the minimum and maximum temperatures was graphically analyzed by plotting the daily data of all the sensors. Outliers and deviations were visually detected and eliminated of the data base. A good specific knowledge of field conditions was taken into consideration as not to delete extreme data recorded by specific sensors.

Since 2017, and due to the large number of data generated by the two probes installed in each solar radiation shield, a new methodology was used to streamline data processing. The absolute deviation from median was calculated for each sensor on each day. Then, the median absolute deviation (MAD) and its

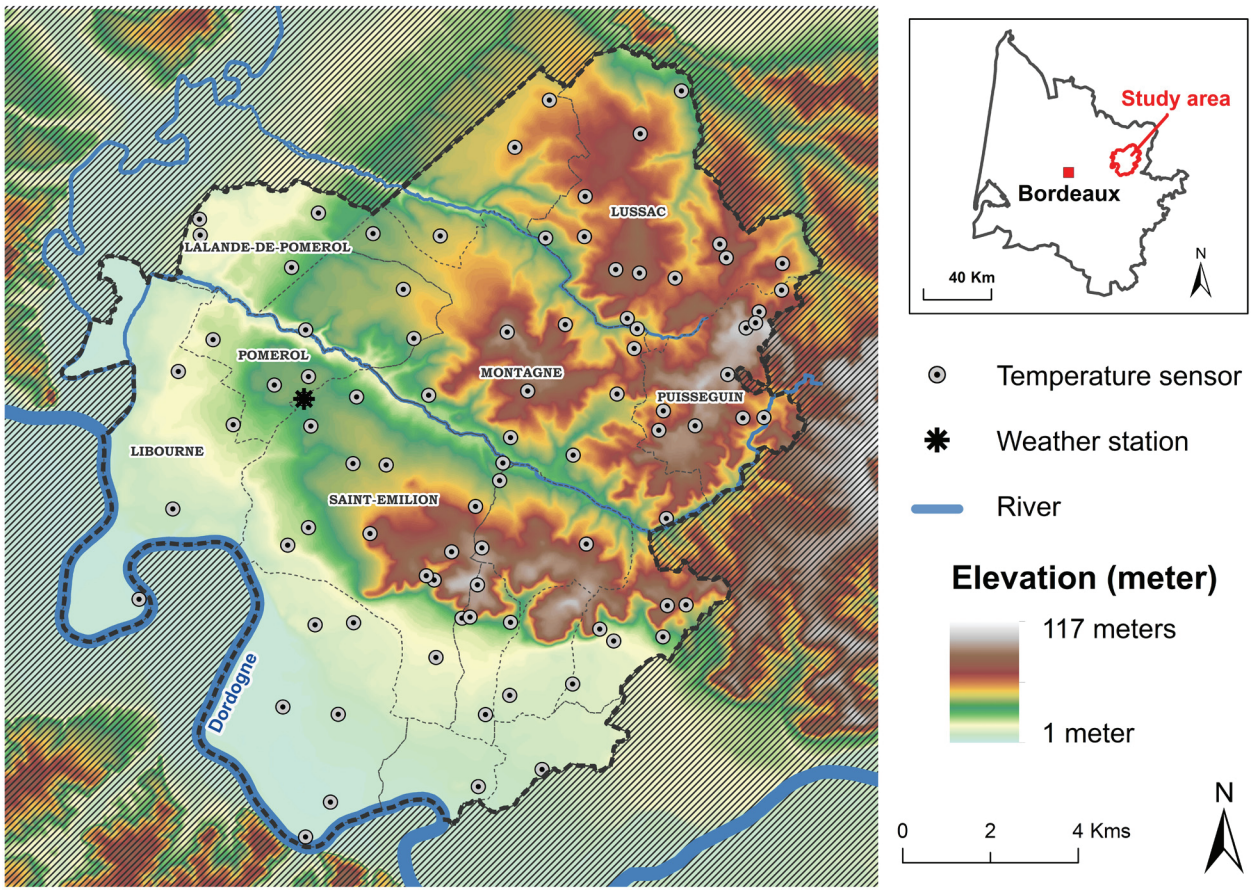

FIGURE 1 | Location of temperature sensors and the weather station on a digital elevation model (IGN, National Geographical Institution, France).

lower and upper bounds were calculated (Leys et al., 2013). MAD was multiplied by a constant (1.4826) linked to the assumption of normality of the data, to avoid the errors induced by outliers (Rousseeuw and Croux, 1993). Initial data were deleted if they were higher than the upper bound or lower than the lower bound calculated for each day. When no significant differences are observed, daily temperatures of both probes were averaged. This methodology was completed by plotting final data to visually detect remaining outliers and by controlling deleted data by comparing it to the initial data recorded by both probes.

For spatial modeling, outlier data were not recreated. For all other treatments, missing data were replaced, except when too much data was missing. If a temperature sensor has less than 30% missing data over the year and less than 20% over the growing season (April 1–September 30), then the data are recreated. For all days where the sensor produced values, deviation is calculated compared to the average of all other sensors of the network. This coefficient is subsequently applied to recreate daily missing data.

Weather Station

Meteorological data from the weather station of Météo-France, located in the Saint-Émilion area (**Figure 1**) were used to regionally characterize the studied years (2012–2018) (**Figure 2**). To compare the temperatures registered by this weather station and those recorded by the Tinytag data loggers located in vine parcels close to the canopy, a Tinytag data logger was set up in a vineyard plot at 4 meters distance to this weather station at the end of 2015.

Bioclimatic Indices

The Winkler degree day summation (Winkler, 1974) was used in this study, as it is well adapted to study the influence of temperature on vine development. This index is based on the sum of mean temperatures above 10°C, from 1 April to 31 October. Because temperatures were measured inside the canopy in this project, and not in a weather station as in Winkler (1974), this index is referred to a canopy Winkler index (CWI) (de Rességuier et al., 2018). CWI is based on the same formula as the Winkler index, the difference is due to the location where temperatures were recorded.

The GFV model (Parker et al., 2011, 2013) was created to simulate the occurrence of mid-flowering and mid-véraison from temperature data for a wide range of grape varieties. This model is based on a sum of daily mean temperatures above 0°C cumulated from the 60th day of the year (DOY).

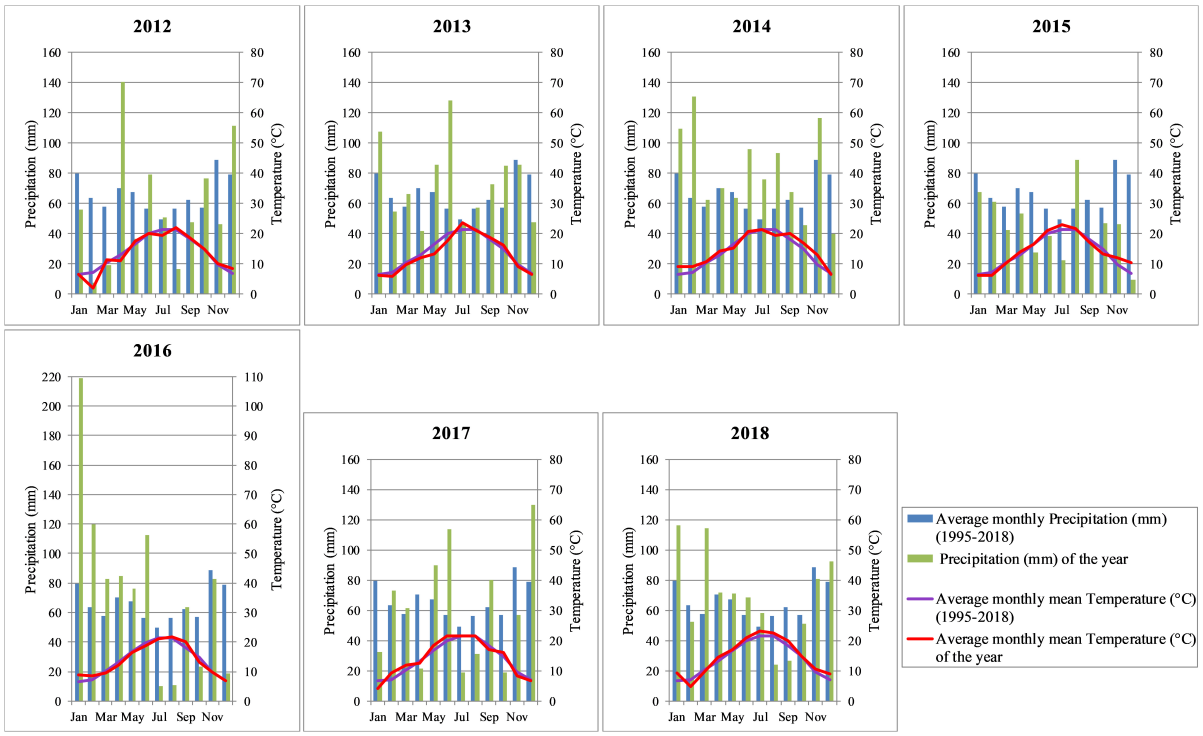

FIGURE 2 | Annual distribution of temperature and rainfall from 2012 to 2018, in comparison to historical period (1995–2018), from the Saint-Émilion Météo-France weather station.

The date of mid-flowering and mid-véraison are determined when the thermal sum reaches a threshold value specific for each grapevine variety. The threshold values for Merlot are 1269 degree-days for mid-flowering and 2636 degree-days for mid-véraison, respectively (Parker et al., 2013). The dates that these threshold values were reached for flowering and véraison were calculated with data from the 90 temperature sensors. Results were compared to real phenology observations from 2012 through 2018 (excluding 2017 when a spring frost event severely damaged vine vegetation over the area).

Vine Development Monitoring

To determine the impact of spatial temperature variability on vine development, the major phenological stages (mid-budbreak, mid-flowering, and mid-véraison) were measured for Merlot from 2013 through 2018 on blocks of 20 vines each at 60 locations near temperature sensors. Only 17 such blocks were surveyed during the 2012 vintage when the project was under development. The specific day when 50 percent of vine organs reached stage "C" for budbreak, stage "I" for flowering and stage "M" for véraison was recorded (Baggiolini, 1952; Destrac-Irvine et al., 2019). The 2017 vintage was excluded from the data analysis, because of frost damage which affected 80% of the reference plots.

Grape maturity dynamics were monitored on 18 blocks chosen to be representative of the thermal variability identified inside the study site. Every week, starting at véraison, grape berries were sampled and major grape metabolites were measured (Destrac et al., 2015). Maturity, which is highly dependent on winegrower decisions and intended wine style, is a phenological stage not easy to assess. Hence, the day when sugar content in grape berries reached 200 g/L was used as a proxy to characterize a theoretical maturity in order to compare the plots.

Environmental Co-variables

Spatial temperature variability at the local scale is influenced by topography-related parameters (Carrega, 1994; Beltrando, 2000; Scherrer and Körner, 2011; Quénol and Bonnardot, 2014). A digital elevation model (DEM) provided by National Geographical Institution (IGN, France), with a 25 m horizontal resolution and 1 m vertical resolution, was used to produce raster layers of elevation, slope, and exposure by using the Spatial Analyst Tools from ArcGis software (ESRI, 92195 Meudon, France). Exposure was separated into two components (north/south and east/west) (Bonnefoy, 2013). Given the size of the study area (17 km*18 km), the latitude and the longitude were also calculated from ArcGis software and taken into account as variables. Pixel values of all these environmental variables were extracted at the location of each temperature sensor.

Statistical Analyses

The effect of environmental parameters (elevation, slope, north/south exposure, east/west exposure, latitude, longitude) on

minimum temperatures on 13 March 2012 and 7 April 2012 were investigated by multiple linear regressions.

Average daily mean, minimum, and maximum temperatures over the growing season, CWI, and phenological observations were represented annually by using boxplot graph set up with the package ggplot2 from R software. Daily thermal amplitude on minimum and maximum temperature (years taken together) were represented monthly by using boxplot graph. In the boxplot representation, outliers are represented as dots. They correspond to observations whose values are higher than the value of the third quartile plus 1.5 times the interquartile interval, or less than the value of the first quartile minus 1.5 times the interquartile range.

Average daily mean, minimum, and maximum temperatures over the growing season and CWI were compared per year using a one-way ANOVA. Daily thermal amplitude on minimum and maximum temperature were compared per month using a one-way ANOVA. The data normality was checked using Kolmogorov–Smirnov one-sample tests and homoscedasticity using Bartlett's test. When a significant effect of year on temperature was found, multiple comparisons were conducted to test differences between each year using Tukey's HSD test (R package agricolae).

The effect of environmental parameters on minimum and maximum average daily temperatures and on CWI were analyzed by using linear mixed models (Pinheiro and Bates, 2000), where elevation, slope, north/south exposure, east/west exposure, latitude, and longitude are considered as fixed effects. Year was considered as a random effect accounting for replicates. Two-way interactions between elevation, slope, north/south exposure, and east/west exposure were measured. All analyses were carried out in R version 3.3.1 (R Core Team, 2016) using packages nlme and car.

Daily maps of minimum and maximum temperatures were created using support vector regression model (Le Roux et al., 2017a). Support vector regression is achieved by machine learning (Cortes and Vapnik, 1995) to estimate complex relationship between dependent variables and a series of predictors. The principle is based on automatic identification of a number of N support vectors (issued from data) between which the non-linear regression function will be estimated through a kernel K (here we used the Gaussian kernel). More complete details of the model and map production are provided in Le Roux et al. (2017a).

From pixels of subsequent daily maps created by the model, estimated dates of mid-flowering and mid-veraison were obtained by implementing the GFV model. A map of these phenological stage dates was produced each year and subsequently averaged over study period.

The root-mean-square error (RMSE) was used to compare mid-flowering and mid-véraison observations from each year (2012–18) with the mid-flowering and mid-véraison dates modeled using the GFV model and from maps produced from modeled mid-flowering and mid-véraison dates.

RESULTS

Temperature Variability (2012–2018)

Daily Temperature Amplitude Analysis

The spatial daily amplitude of minimum and maximum temperature (i.e., the difference between the highest and lowest minimum and highest and lowest maximum temperature) over the study area was analyzed from 2012 through 2018. **Figure 3** represents the daily amplitudes per month (average of all years).

Spatial amplitude of minimum temperatures (average of 3.9°C ± 1.7, **Figure 3A**) is greater than for maximum temperature (average of 3.2°C ± 0.9, **Figure 3B**). The amplitude of minimum temperatures can reach 9.8°C on specific days, and the large size of the boxplot reveals important variations of amplitude from day to day (from 1 to 10°C).

Boxplot sizes of daily amplitude of maximum temperature are smaller, which indicated less variation from day to day, except some specific days (outlier points of the graph) (**Figure 3B**). Daily amplitude of maximum temperature presents also a seasonal effect, with greatest amplitude from June to October (a and b ANOVA groups).

The spatial structure of temperatures can be very different from day to day due to the weather, the atmospheric circulation, and the effects of the morphological features and land properties. In general, the spatial variability of temperatures is greater in atmospheric situations with clear skies and light winds. During clear sky days, more solar radiation reaches the surfaces and provokes higher levels of heating. During the night, clear sky induces stronger cooling due to greater long wave radiation emitted from surfaces. The distribution of minimum temperatures on 13 March 2012 was chosen as an example to illustrate the distribution of temperatures during an anticyclonic day, with clear sky conditions and no wind (**Figure 4A**). This weather type leads to large amplitude of minimum temperatures, by promoting cold air to flow into valleys and plains accentuated by long wave radiation emitted from surface. A minimum temperature amplitude of 9.2°C was recorded on this day, and relief is well correlated to this spatial distribution with coolest temperatures in the lowest parts of the valleys and warmest temperatures at the top of the hills on the limestone plateaus. Statistical analysis using multiple linear regressions of the minimum temperature of all the sensors against environmental parameters found elevation to be the most significant. Elevation positively explained 51.7% of the variance and latitude negatively explained 4.2%, for a total of 59% of the variance overall explained by the model. Conversely, a very different spatial minimum temperature distribution was observed on 7 April 2012, which was a cloudy atmospheric depression day with no wind (**Figure 4B**). This weather type reduces spatial thermal amplitude, which was restricted to only 1.1°C over the area. However, relief plays an important role on the minimum temperature distribution. During this day, 66% of the variance is negatively explained by elevation, 2.7% and 2.5% of the variance is positively explained by the east/west exposure and by longitude, respectively, and 1.5% of the variance is negatively explained by north/south exposure, with a total

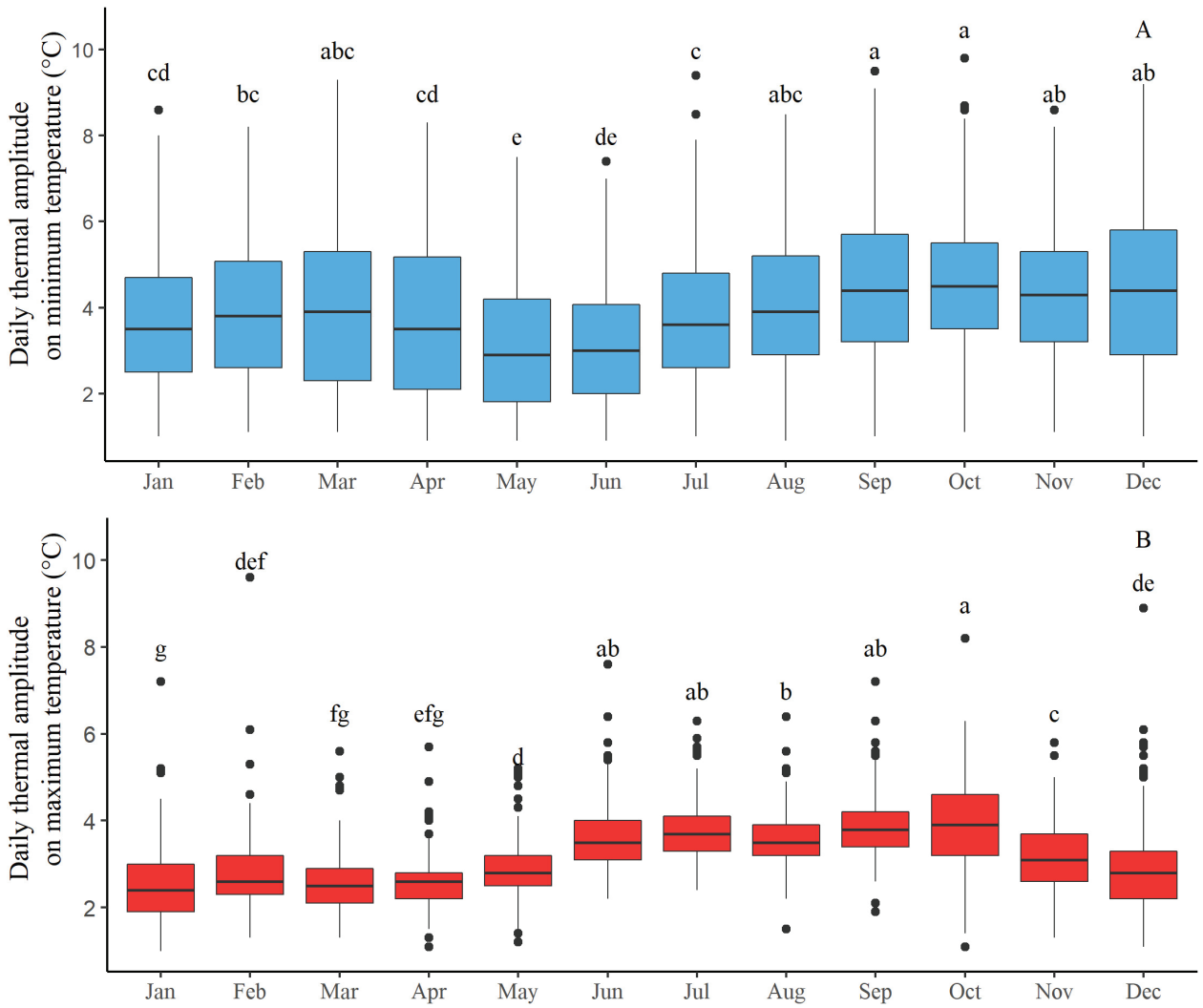

FIGURE 3 | Monthly boxplots of the daily thermal amplitude for **(A)** minimum and **(B)** maximum temperature over the site from 2012 through 2018.

of 76% of the overall variance explained by the multiple linear regression model.

The effects of topography and other local factors are also combined with larger scale factors related to synoptic conditions (Planchon et al., 2015). The atmospheric circulation strongly influenced by the Atlantic Ocean generates a longitudinal gradient with higher temperatures over the western part of the site (Le Roux, 2017).

Temperature Variability Over the Vegetative Season

Average daily mean, minimum, and maximum temperatures were analyzed during the growing season, from 1 April to 30 September during seven consecutive years (2012–2018) (**Figure 5**).

The average mean temperature is around 19.1°C ± 0.6 (with a mean amplitude of 1.5°C ± 0.2) and shows a marked vintage effect: 2018 is the warmest and 2013 the coolest year.

The intra-annual spatial variability, which corresponds to the range of temperatures between the coldest and the warmest sensor, is greater for minimum temperatures (2.6°C ± 0.3 in average over the seven vintages) than for maximum temperatures (2.1°C ± 0.3 in average).

The inter-annual (temporal) variability is greater for mean and maximum temperatures than for minimum temperatures. The multiple comparisons performed after the ANOVA found only three groups for the minimum temperatures in comparison to six and seven groups for the mean and maximum temperatures, respectively. Hence, at this site, the vintage effect is mainly driven by variations in maximum temperatures.

Canopy Winkler Index

In order to improve the characterization of temperature variability, the canopy Winkler degree-days summation was calculated for each sensor in each year (**Figure 6**).

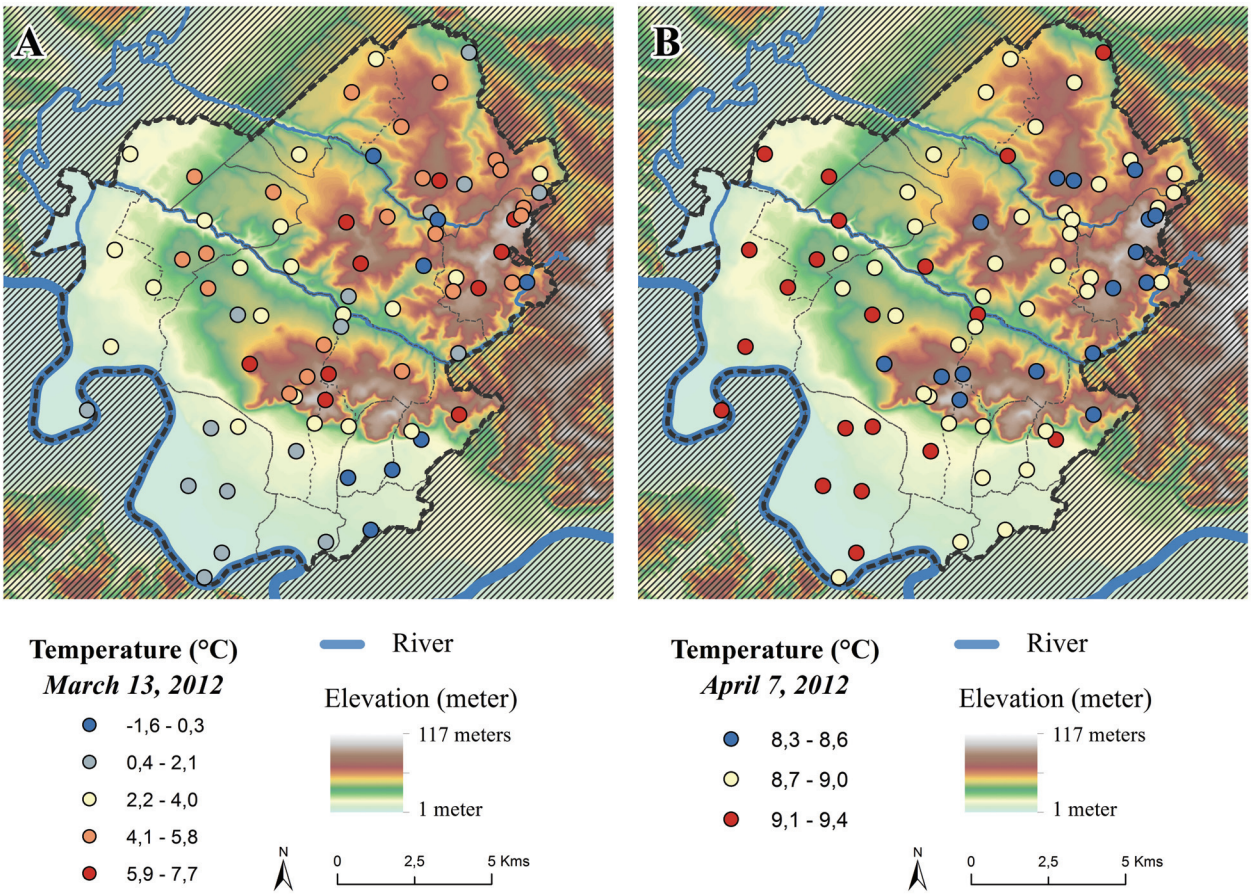

FIGURE 4 | Maps of minimum temperature of 13 March **(A)** and 7 April **(B)** 2012.

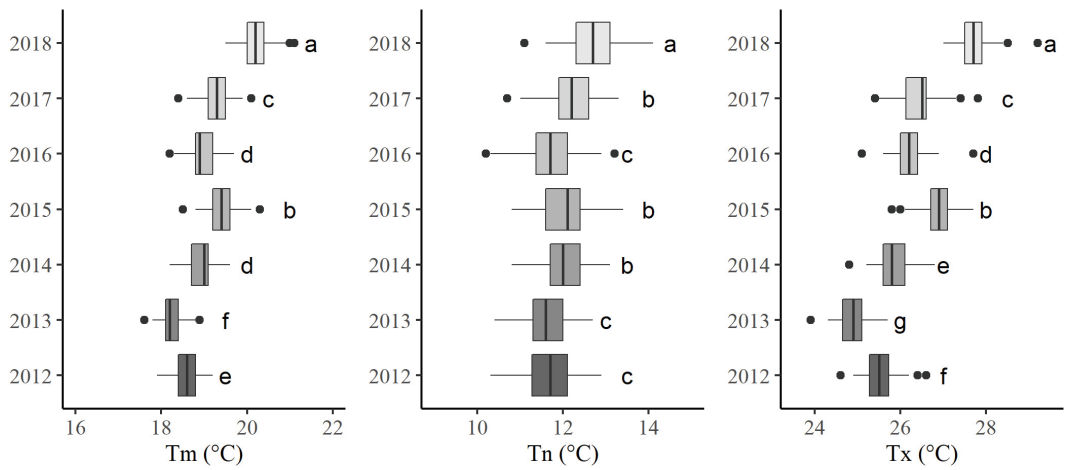

FIGURE 5 | Boxplots of mean (Tm), minimum (Tn), and maximum (Tx) average daily temperatures over the growing season (from 1 April to 30 September) from 2012 through 2018. Different letters indicate significant differences between years (at $P < 0.05$).

An important vintage effect was highlighted with 2018 being the warmest and 2013 the coolest vintages, with CWI of 2041 degree-days ± 72.6 and 1735 degree-days ± 55.4, respectively. The spatial amplitude was also substantial, with an average of 320 degree-days ± 41.7 over the 7 years studied.

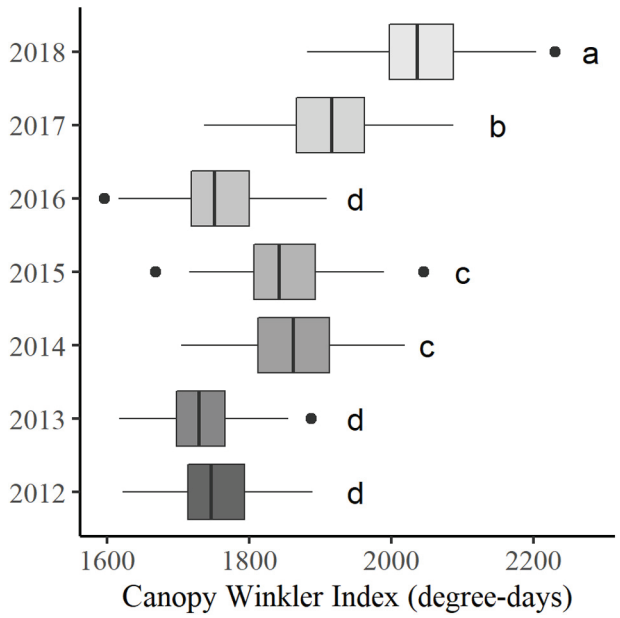

FIGURE 6 | Boxplots of canopy Winkler index from 2012 through 2018. Different letters indicate significant differences between years (at $P < 0.05$).

Temperatures Modeling

Spatial Modeling of Daily Temperatures

Minimum and maximum temperature maps were created for every day of the period under study (2012–2018). **Figure 7** represents the spatial temperature distribution of 2 days with extreme conditions. 7 March 2015 was an anticyclonic day with clear weather without wind and rain, where temperatures dropped below 0°C in the areas most sensitive to cold air accumulation. 19 July 2016 was characterized by warm and dry weather, where the maximum temperatures of some areas located in the western and eastern parts of this area exceeded 40°C.

Spatial Distribution of Minimum and Maximum Temperatures During the Vegetative Season

Daily maps of minimum and maximum temperature were integrated over the growing season for each year. Independently of the vintage effect, a recurring spatial structure was shown (Le Roux et al., 2017b). It was therefore decided to average temperature maps over the duration of the study to quantify the temperature distribution for the purpose of producing a temperature zoning.

The analysis of the average minimum and maximum temperature maps over the study area shows a high spatial variability at this scale. For minimum temperatures, the sectors

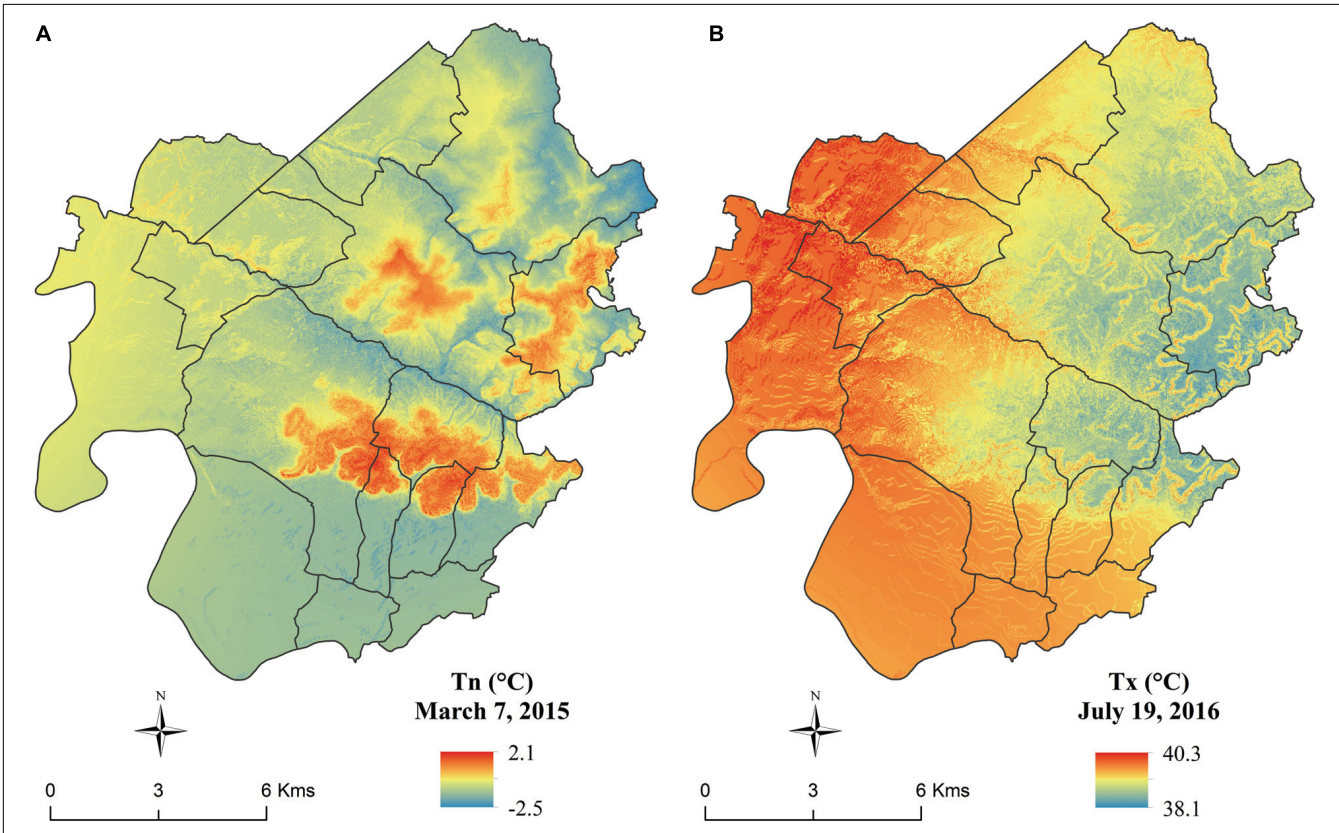

FIGURE 7 | Spatial distribution of minimum temperature on 7 March 2015 **(A)** and of maximum temperature on 19 July 2016 **(B)**.

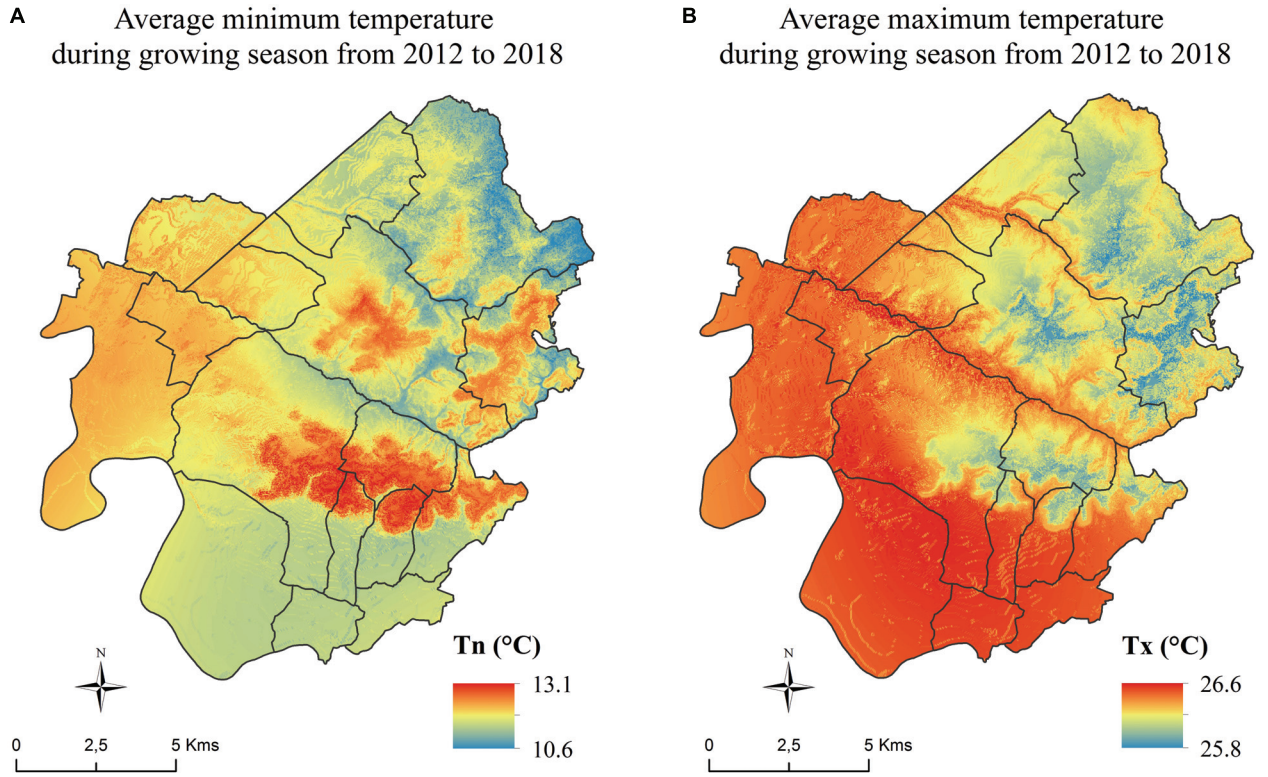

FIGURE 8 | Spatial distribution of average **(A)** minimum and **(B)** maximum temperatures during the growing season (1 April to 30 September) from 2012 through 2018.

with the highest altitudes (limestone plateaus of Saint-Émilion, Montagne, Puisseguin, and Lussac), as well as those on south exposed slopes, correspond to the highest minimum temperatures (**Figure 8A**). Conversely, the lowest sectors (the Dordogne alluvial plain and the bottoms of the valleys) are associated with the lowest temperatures. The western part of the area (Libourne, Pomerol, Lalande-de-Pomerol) did not follow this distribution and minimum temperatures are slightly above average, while the altitudes are relatively low and the slopes close to zero.

For the spatial distribution of maximum temperatures, the opposite spatial pattern is observed: the warmest temperatures are recorded at low altitudes and coolest temperatures at high altitudes. High maximum temperatures are also recorded in the western part of the area (appellations Pomerol, Lalande de Pomerol, and the commune of Libourne, **Figure 8B**). The spatial thermal amplitude of the mean maximum temperatures is smaller compared to the minimum temperatures.

Finally, the areas with the greatest thermal amplitude between minimum and maximum temperatures are located at the bottom of the hills while the parcels located in the highest positions show smaller thermal amplitude.

Spatial Distribution of Canopy Winkler Index

The spatial distribution of the CWI (**Figure 9**) shows a spatial structure which is linked to the relief. The limestone plateau of Saint-Émilion and its south facing slopes are the warmest parts of the area. The north-east of the area is the coldest sector. The Dordogne alluvial plain and the bottom of the valleys are cooler. Another warm part of the region, not specifically linked to the topography, is the western part of the area around the town of Libourne, including Pomerol and Lalande dePomerol.

Environmental Factors Explaining Temperature Distribution

A statistical analysis was implemented to select the geomorphological co-variables which drive temperature distribution (**Table 1**). The main factors impacting minimum temperature over the vegetative season are elevation, longitude, slope, and latitude. Tn increased with elevation and the percentage of slope, and decreased from west to east and from south to north. Significant, but less important effects were found for exposure variables. The effect of elevation was, however, contingent on slope, and exposure parameters. For example, the negative coefficient parameter estimate for the interaction between elevation and slope indicated that the increase of minimum temperature with elevation is stronger in very steep vineyards than in parcels with low declivity.

Regarding maximum temperatures, the main effect is elevation. Tx decreased with elevation and increased with percentage of slope. The effect of elevation was, however, contingent on slope, on south/north exposure, and on west/east

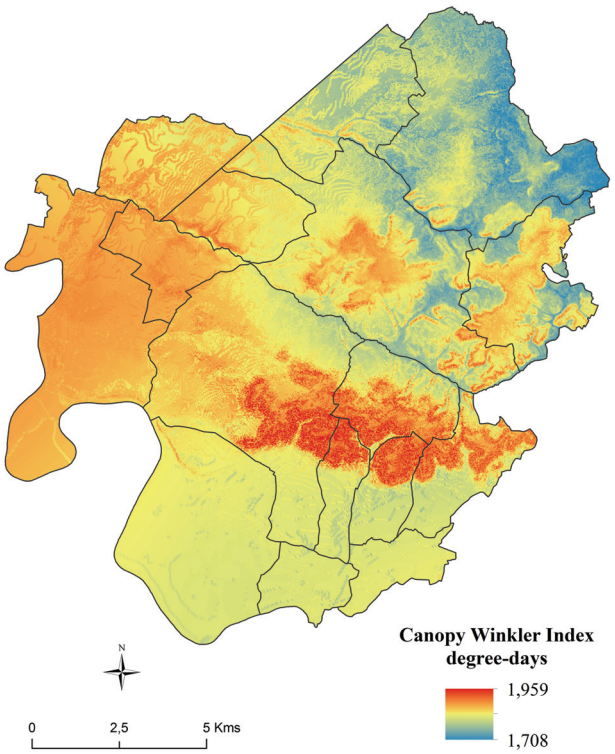

FIGURE 9 | Spatial distribution of average Canopy Winkler Index (2012–2018).

exposure. Regarding the interaction between elevation and west/east exposure, the positive coefficient suggested that the effect of elevation on maximum temperatures was lower in east facing parcels.

Canopy Winkler index increased with elevation, slope, and decreased with north/south exposure, longitude, and latitude. The effect of elevation was concomitant with slope. The negative coefficient parameter for the interaction showed that the increase of CWI with elevation was higher in steep vineyards than for parcels with low declivity.

Relationship Between Vine Development and Temperature

Phenological Observations

Phenology monitoring revealed the importance of the vintage effect: the warmer meteorological conditions of 2018 and 2015 advanced the timing of phenological stages compared to the cool year 2013 (**Figure 10**). The duration of phenophases between the phenological stages was variable from year to year. Some vintages, like 2016, can have an early budbreak due to high temperatures during the beginning of the year followed by a late flowering and véraison, because of relatively lower temperatures later in the growing season (**Figure 2**).

The intra-annual variability was highlighted in this study. An average window of about 19 days ± 7.7 was recorded for budbreak, 9 days ± 2.9 for flowering, 13 days ± 4.5 for véraison, and 25 days ± 11.3 for theoretical maturity (200 g/L of sugar content). The standard deviation showed more variation between years for budbreak and maturity, which is due to meteorological conditions affecting duration during these phenophases. For example, the maturity of the 2013 vintage was impacted by the poor ripening conditions that led to a delay in maturity. The 2012 budbreak was also affected by the cool temperatures at the beginning of the year and the rainy weather in April, which increased the duration of this stage.

The Timing of Mid-Flowering and Mid-Véraison Modeled by Means of the GFV Model

The GFV model was developed to predict the timing of flowering and véraison for a wide range of grapevine varieties. One of the objectives of this study was to produce occurrence maps of mid-flowering and mid-véraison dates by using the GFV model. To do so, the prediction accuracy of the GFV model for Merlot at this local scale needed to be validated first.

Temperature data correction

The GVF model was developed with data collected at regular weather stations. Data recorded by the Tinytag thermistor probe installed inside the vegetation were matched with the temperatures of the Météo-France weather station in order to compare the recorded temperatures.

Temperatures registered by both systems are different for minimum and maximum temperatures (**Figure 11**). The gap on minimum temperatures (average of −0.2°C ± 0.3) is less important compared to maximum temperatures (average of 1.2°C ± 0.7). A seasonal effect was also observed for maximum temperatures with greater differences during the vegetative season.

Considering these differences, it appeared necessary to correct the data collected with the Tinytag data loggers in order to use the published GFV model parameters. A linear correction was shown to be satisfactory for minimum and maximum temperatures (**Figures 12A,B**).

The daily minimum and maximum temperature data recorded by Tinytag data loggers from 2012 to 2018 were corrected using these linear models in order to validate the GFV model.

Validation of GFV model

The GFV model was used to simulate the occurrence of mid-flowering and the mid-véraison for Merlot from corrected data of the 90 temperature sensors. Results were compared to the phenological observations to validate the performance of the GFV model at the scale of this site.

Figure 13 represents the differences between the observation and the GFV prediction for mid-flowering (A) and mid-véraison (B). The GFV model performed well at this local scale, with an accuracy of 4 days for mid-flowering including the 2013 vintage, which presents less precise results. Similar results were found for mid-véraison, the majority of the prediction errors are lower than 5 days, with an exception for the 2013 vintage. 2013 is a particular vintage with a very fresh and rainy beginning of the year (**Figure 2**). Flowering was affected by these adverse meteorological conditions, inducing poor fertilization which provoked coulure and millerandage and heterogeneous maturity. Storms in late July and early August provided unlimited water

TABLE 1 | Summary of Linear Mixed-Models testing the effect of elevation, slope, exposure, latitude, and longitude on maximum temperature, minimum temperature, and Canopy Winkler index.

	Maximum temperature (°C)			Minimum temperature (°C)			Canopy Winkler index (degree-days)		
	Estimate	Std. error	χ^2-value	Estimate	Std. error	χ^2-value	Estimate	Std. error	χ^2-value
Elevation (m)	−6.9E-03	9.0E-04	**126.0 (<0.001)**	2.3E-02	1.0E-03	**681.9 (<0.001)**	1.85E+00	1.37E-01	**174.6 (<0.001)**
Slope (%)	6.7E-02	1.6E-02	**16.6 (<0.001)**	1.1E-01	1.8E-02	**108.0 (<0.001)**	2.03E+01	2.38E+00	**133.0 (<0.001)**
South/north exposure	2.0E-01	5.1E-02	0.1 (0.7)	−2.5E-01	5.8E-02	**6.4 (0.01)**	−6.78E+00	3.07E+00	**4.9 (0.03)**
West/east exposure	−4.1E-01	6.8E-02	7.3 (0.007)	2.9E-01	7.6E-02	**14.8 (<0.001)**	3.28E+00	3.69E+00	0.8 (0.4)
Longitude (m)	−6.2E-07	5.0E-06	0.02 (0.9)	−1.3E-04	5.0E-06	**574.1 (<0.001)**	−1.00E-02	1.00E-03	**377.3 (<0.001)**
Latitude (m)	−1.0E-05	4.0E-06	1.9 (0.2)	−4.0E-05	5.0E-06	**68.0 (<0.001)**	−4.85E-03	1.00E-03	**62.7 (<0.001)**
Elevation (m) * slope (%)	−1.0E-03	3.2E-04	**10.3 (0.001)**	−1.2E-03	3.6E-04	**10.3 (0.001)**	−2.60E-01	4.90E-02	**28.8 (<0.001)**
Elevation (m) * south/north exposure	−3.5E-03	8.4E-04	**17.2 (<0.001)**	3.5E-03	9.5E-04	**13.2 (<0.001)**	/	/	/
Elevation (m) * west/east exposure	7.0E-03	1.3E-03	**29.7 (<0.001)**	−3.7E-03	1.4E-03	**6.6 (0.01)**	/	/	/

P-values are indicated within brackets and significant effects are shown in bold.

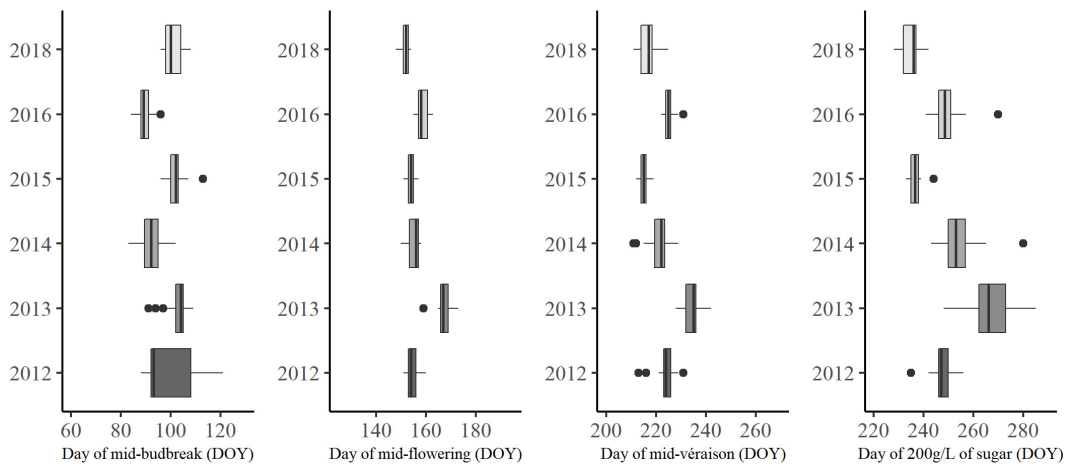

FIGURE 10 | Boxplots of observed phenological stages from 2012 through 2018.

supply to the vines, causing continued vegetative growth and late and untypical ripening conditions.

Root-mean-square error was calculated for each year and phenological stage. Average RMSE (**Table 2**) is 2.2 days for flowering and 3.6 days for véraison, which shows that the GFV model is able to predict flowering and véraison with great accuracy at this scale. By comparison, RMSE of the GFV model for Merlot published in Parker et al. (2013) was 5.6 days for flowering and 6.6 days for véraison.

Spatial modeling of the timing of mid-flowering and mid-véraison (2012–2018)

Maps of spatial distribution of the occurrence of mid-flowering and mid-véraison stages averaged over the period 2012–2018 were created by using the daily maps of corrected temperature and the parameters of GFV model for Merlot (**Figures 14A,B**).

The spatial structure of the maps of both stages is similar and in adequacy with the spatial structure of CWI. The limestone plateaus, the south facing slopes, and the western part of the area have the earliest phenology overall. The northern and eastern part of the area, as well as the bottom of the valley (especially those located in the north eastern part of the area) show delayed phenology. The largest amplitude was found for the véraison stage, which is similar to the results from phenology monitoring (**Figure 10**). However, the spatial modeling reduces the amplitude across the area, compared to real observations from 9 to 6 days for mid-flowering dates and 13 to 9 days for mid-véraison dates.

Validation of phenological maps

To validate the phenology maps, an extraction of the pixel value at the location of each temperature sensor

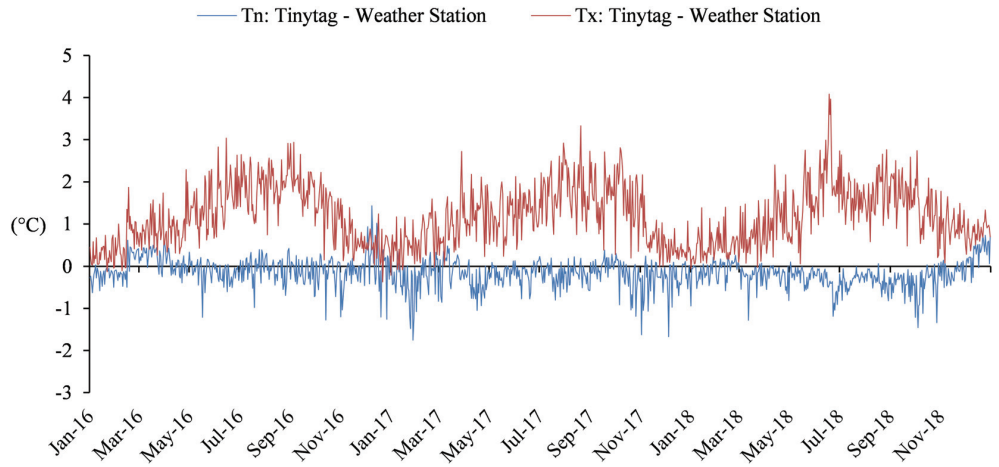

FIGURE 11 | Comparison between temperatures recorded by Tinytag thermistor probe and the weather station of Saint-Émilion on daily minimum and maximum temperatures from 2016 through 2018.

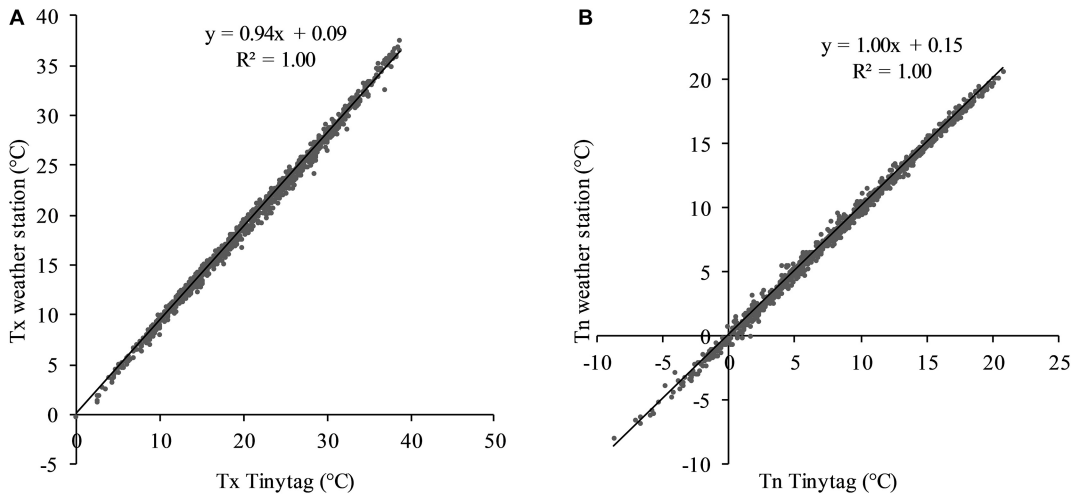

FIGURE 12 | Relationships between minimum temperatures measured by Tinytag thermistor probe and weather station **(A)** and relationship between maximum temperatures measured by Tinytag thermistor probe and weather station **(B)**. Data cover 2016–2018.

was carried out for each year, for flowering and véraison. Results were compared to phenology observations. The average RMSE is 2.3 days ± 0.7 for flowering and 3.5 days ± 1.5 for véraison, which corresponds to a similar result for observations of flowering and véraison compared to modeled flowering and véraison dates calculated directly using temperature data collected with the thermistor probes (**Table 2**). Hence, no accuracy loss was detected with the spatial modeling procedure.

DISCUSSION

Spatio-Temporal Temperature Analysis

Based on a large unprecedented dataset obtained by a high density network of temperature observations, important spatial amplitude of seasonal temperatures over this area was observed. An average of 320 degree-days of amplitude over the 7 years studied was found for CWI.

This study also underlines large spatial amplitude on minimum temperatures during the vegetative season and great inter-annual variation of maximum temperatures, which impacts the vintage effect. Hence, spatial temperature variability was more driven by minimum temperatures, while temporal (i.e., year-to-year) temperature variability was more driven by maximum temperatures. Previous studies investigating temperatures at the vineyard scale have also shown great spatial temperature variability (Quénol et al., 2004; Bonnardot et al., 2012; Bonnefoy, 2013; Cuccia, 2013). These studies highlighted the impact of relief or local parameters such as water bodies on temperature distribution. This study shows not only the impact of relief,

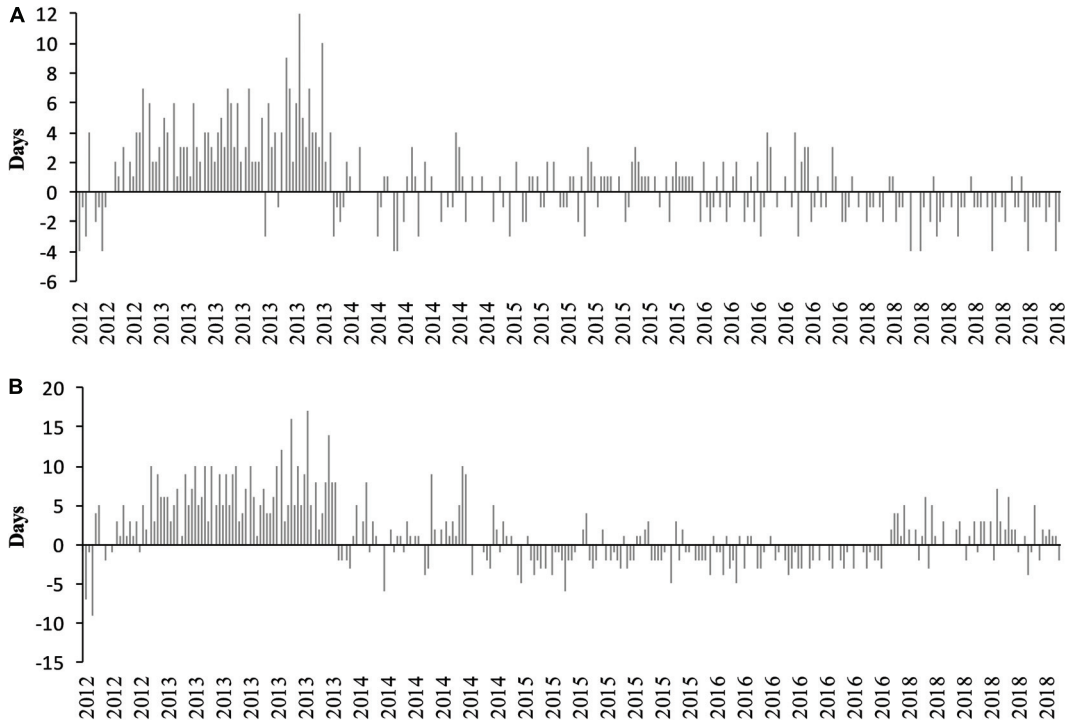

FIGURE 13 | Differences between observed and predicted dates by using the GFV model for mid-flowering **(A)** and mid-véraison **(B)** from 2012 through 2018.

but also latitude and longitude. It confirms that great spatial thermal amplitude at local scale is well connected with local environment, as was also shown by Quénol et al. (2014) and Neethling et al. (2019).

Daily temperature analysis showed large spatial amplitude, especially on minimum temperatures. Spatial structure varies from day to day depending on the weather type, which confirms findings of other studies at the local scale (Madelin and Beltrando, 2005). The distribution of temperatures varies according to the atmospheric situation. Lower scale temperatures are also dependent on higher climatic scales. To explain the distribution of daily temperatures, it is necessary to study synoptic situations and their consequences on temperature distributions at the local scale. A preliminary study, based on data from this network over the period 2012–2015, showed an influence of weather types (Cantat et al., 2012) and atmospheric circulation patterns (Hess and Brezowsky, 1952) on the spatial and temporal daily variability (Eveno et al., 2016). This methodology was previously used is several studies to identify spatial climate variability (Douvinet et al., 2009; Planchon et al., 2009). Preliminary results in our study site show that great amplitude on minimum and maximum temperatures seems to result from northwest/north circulation, but also that warm weather induces great daily spatial amplitude on minimum temperature. These results need to be confirmed over the duration of the project with the use of appropriate statistical tools.

Another approach to assess the impact of weather conditions on spatial temperature variability is to classify daily temperature maps based on statistical criteria. In a second step, synoptic atmospheric conditions (wind direction and atmospheric pressure) of each cluster can be determined. This approach was tested on the 2014 data of this study site (Le Roux et al., 2017b). A classification in nine nodes of the spatial distribution of temperature was obtained. To determine the average daily atmospheric conditions of each node, the outputs of the regional model (Weather Research and Forecasting) were used. Spatial variability of temperatures was analyzed according to the atmospheric situation and allowed a better understanding of the results regarding the distribution of the temperatures over the study area. This approach was, however, carried out for only 1 year, and needs to be conducted over a longer period of time to confirm the observed relationships with increased statistical power.

In the future, it will be interesting to combine these two different approaches to better understand the influence of the weather type and the atmospheric situation on spatial temperature distribution at the local scale.

Relationships Between Vine Development and Temperature

It is well established that temperature is a major driver of plant phenology (Chuine et al., 2013). Most studies assessing the relationship between temperatures and phenology are based on point data (i.e., data obtained in specific locations). Spatial modeling of phenological stages has been implemented,

TABLE 2 | RMSE calculated to compare mid-flowering and mid-véraison observations for each year (2012–18) with mid-flowering and mid-véraison dates modeled using corrected temperature data and from maps produced using modeled mid-flowering and mid-véraison dates.

Years	Number of flowering observations	RMSE (days) of flowering observations/flowering modeled from corrected temperatures by GFV	RMSE (days) of flowering observations/flowering dates extracted from a map created with modeled flowering dates using GFV	Number of véraison observations	RMSE (days) of véraison observations/véraison modeled from corrected temperatures by GFV	RMSE (days) of véraison observations/véraison dates extracted from a map created with modeled véraison dates using GFV
2012	17	2.2	2.3	17	3.7	3.7
2013	64	4.7	3.6	63	7.6	6.2
2014	59	1.7	2.4	59	3.4	3.5
2015	60	1.4	1.5	60	2.4	2.6
2016	58	1.8	1.8	60	2.2	2.3
2018	62	1.7	2.4	55	2.6	2.4
Mean (2012–18)		2.2	2.3		3.6	3.5
Std (2012–18)		1.2	0.7		2.0	1.5

but only at large scale (Fraga et al., 2016; Sgubin et al., 2018). Our aim was to produce local scale maps of the occurrence of phenological stages. To do so, our spatial temperature modeling was coupled to phenology modeling. A high number of phenology observations were used as a validation dataset.

The GFV model (Parker et al., 2011) used to assess mid-flowering and mid-véraison dates was developed with data collected by regular weather stations. In this study, temperature data were recorded by Tinytag thermistor probes installed inside the vegetation. Hence, it was necessary to compare obtained data with temperatures recorded by a regular weather station. Temperatures registered by both systems are different for minimum and maximum temperatures. This result underlines influences of the local environment (vine parcel, canopy), but also an impact of intrinsic characteristics of measurement equipment (type of sensor, solar shield) on recorded temperatures. In order to use the published GFV model parameters (Parker et al., 2013) on the data collected with the Tinytag data loggers, temperatures collected in this study were corrected by means of a linear regression.

Several hundred real phenology observations allowed validation of the GFV model at this scale (RMSE = 2.2 and 3.6 days for mid-flowering and mid-véraison, respectively). Model performances were poorer in 2013, when unfavorable meteorological conditions during flowering induced fertilization problems (RMSE = 4.7 and 7.6 days for mid-flowering and mid-véraison, respectively). By coupling GFV and temperature models, maps of flowering and véraison were created and validated over this study area. These maps are highly accurate, as shown by comparing modeled phenology dates extracted from the maps with phenology observations (RMSE = 2.3 and 3.5 days for mid-flowering and mid-véraison, respectively). To improve precision, it would be interesting to develop site-specific phenology models, based on phenological observations and temperature data obtained over this area.

The GFV model performs well in current meteorological conditions, but it may be less accurate under much warmer conditions, because it is not capped for extreme temperatures. In a context of climate warming, and considering that phenology is the first biological indicator of climate change (Menzel et al., 2006), it will be interesting to also test models like the one created by Wang and Engel (1998), which identifies an optimal temperature and a critical threshold temperature above which plant development is stopped. This type of model is certainly more accurate to project phenology evolution over this site in extreme scenarios of climate change (RPC 8.5, end on the century).

To evaluate the impact of climate change on vine development, several studies were carried out in different areas (Cuccia, 2013; De Cortázar-Atauri et al., 2017; Alikadic et al., 2019). Coupling temperature projections under various climate change scenarios (IPCC, 2013) with spatial phenology modeling developed in this study will allow the creation of maps projecting phenological stages over this site.

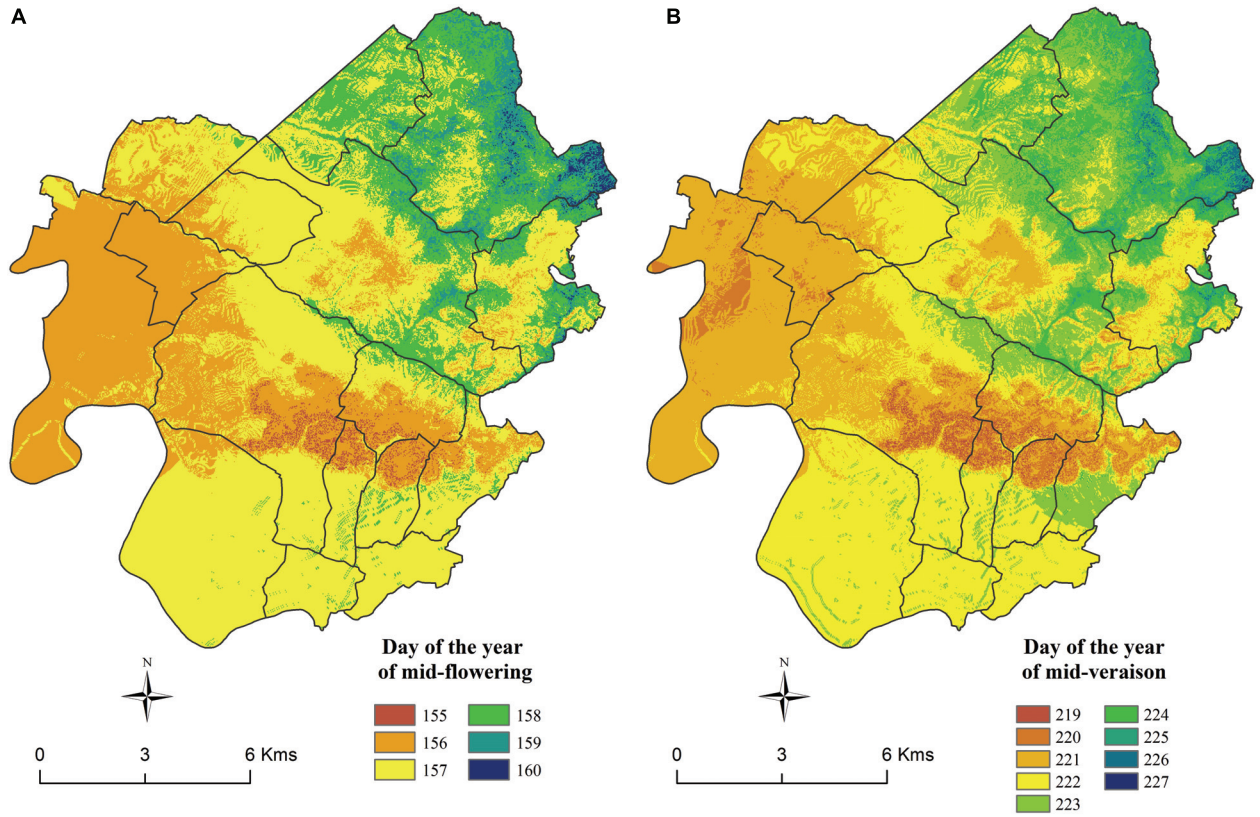

FIGURE 14 | Maps of the average modeled occurrences of **(A)** mid-flowering and **(B)** mid-véraison stages (2012–2018) for Merlot.

Comparing Amplitude of Temperature and Phenology Observations With Amplitude of Temperature and Phenology on Maps Obtained by Spatial Modeling

In our dataset, amplitude (i.e., the difference recorded by the coldest and warmest sensors) is 2.6°C for Tn, 2.1°C for Tx and 320 degree-days for CWI (average 2012–2018). On the maps obtained by spatial modeling, this amplitude is very similar for Tn with 2.5°C but is reduced to 0.8°C for Tx and 251 degree-days for CWI (average 2012–2018). Similar reduction is shown for phenological stages (from 9 to 6 days for mid-flowering dates and 13 to 9 days for mid-véraison dates).

The spatial modeling of the minimum temperature over the vegetative season is very accurate and the temperature ranges and the amplitudes are very close to the recorded temperatures. On the other hand, there is a sharp reduction of modeled amplitudes of maximum temperature. Visualization of the measured maximum temperature distribution over the different growing seasons (**Figure 5**) shows that the amplitudes are often extended by extreme points, which is not the case for the minimum temperature distribution. Extreme points on maximum temperature are often located in valleys close to vegetation like trees or hedges. Regarding the extreme coldest maximum temperatures, they are often located in higher elevation areas where environmental parameters favor air circulation and consequently temperature reduction.

The few extreme points influencing the large measured amplitude of maximum vegetative season temperature over the study area represent only a small weight in the spatial modeling and explain this reduction of amplitude after spatial modeling. The maximum temperature can be influenced by specific local environments like vegetation or wind, which are not taken into account in the modeling because they are not well represented. At this scale of modeling, and taking into account latitude/longitude and the relief parameters as co-variables, extreme points are prevented from having an important impact on spatial modeling.

It can be assumed that this amplitude reduction on the modeled maximum temperatures induced subsequently the loss of amplitude on the modeled CWI, which is created from compilation of daily modeled maps of Tn and Tx.

The reduction of amplitude of modeled phenology, compared to observed phenology, can result from decreased modeled temperature amplitude. Specific plant responses induced by factors other than temperature, like plant water or mineral status, plant age, clone or root-stock can, however, also impacted measured amplitude. These biotic and abiotic factors are not taken into account by the phenology models used in this research.

Relationships Between Day–Night Temperature Amplitude and Wine Quality

High temperature amplitude (i.e., differences between day and night) during grape ripening is often considered to be a wine quality enhancing factor. This idea is frequently developed in popular wine books, and thermal amplitude during grape ripening is sometimes included in climatic characterization of wine producing areas (among other references see Montes et al., 2012). It is thought to increase secondary metabolites (phenolic compounds and aromas) in grapes and wines, although scientific evidence on this topic is scarce and somewhat contradictory. In one of the earlier references, Kliewer and Torres (1972) found that high anthocyanin accumulation in grape berries was related to *low* thermal amplitude. In contradiction with this, Mori et al. (2007) showed that, for a given day temperature, anthocyanins in grapes were higher when night temperature was low (i.e., in the case of higher day–night thermal amplitude). Regarding other phenolic compounds, Kliewer and Torres (1972) did not find a strong temperature amplitude effect on flavonols and so did Cohen et al. (2012) on proanthocyanidins. Over our study site, we found low temperature amplitudes on the limestone plateaus, where some of the finest wines of the area are produced, and high temperature amplitude in the valleys, known for producing entry level wine quality. This observation does not support the idea that high temperature amplitude is associated to high wine quality. Similar results were found by Bois et al. (2018) who recorded low day–night temperature amplitude in the Médoc area close to the Gironde Estuary (Bordeaux, France), where some of the finest Bordeaux wines are produced. Hence, inside the Bordeaux production area, high day–night temperature amplitude does not seem to be associated to high wine quality. More research is, however, required on this topic. Thermal amplitude is related to elevation and the proximity of water bodies, and so is soil distribution. It is possible that, in the case of the Bordeaux area, the effect of soil type overrules a potential impact of thermal amplitude. It is also possible that, independently from thermal amplitude, lower maximum temperatures promote grape quality potential: high temperatures induce cooked fruit aromas which are not associated with premium wine quality (Allamy et al., 2017; Pons et al., 2017).

Terroir Characterization at Local Scale for a Better Adaptation of Current and Future Technical Management Strategies

Terroir is a concept based on the observation that wine quality and typicity are impacted by the physical and biological environment (van Leeuwen and Seguin, 2006). Major factors of the terroir effect are climate and soil (van Leeuwen et al., 2004; Bodin and Morlat, 2006; Morlat and Bodin, 2006; Jones, 2018). Terroir zoning is important for winegrowers in order to optimize the potential of their terroir by adapting plant material (rootstock and variety), training system, vineyard floor management, and harvest decisions to local climate and soil conditions (Reynolds et al., 2007; Vaudour et al., 2015; van Leeuwen and de Rességuier, 2018). Detailed soil maps are available for many winegrowing regions, including this study area (van Leeuwen et al., 1989; Swinchatt et al., 2018).

A previous study over the Bordeaux area produced temperature maps at 50 m of resolution (Bois, 2007; Bois et al., 2018). The results presented in our study increase the resolution of temperature mapping in a specific area of the Bordeaux wine region. To obtain this high resolution (25 m), a non-linear spatial model was developed based on the temperature data recorded by a high density temperature sensor network scale (Le Roux et al., 2017a). Maps of the different temperature indicators and agro-climatic indices, as well as maps of phenological stages, were produced in this research over the duration of the project and are well adapted to be used by large estates or cooperative cellars. These maps, and knowledge of local parameters involved in spatial temperature distribution, will help wine growers to better adapt plant material and viticultural practices. It will also allow them to determine harvest dates with increased precision. At very local scale, however, landscape features such as hedges or trees can influence temperature distribution (Quénol et al., 2014). These parameters have not been taken into account in our models. Hence, the interpretation of the temperature maps in these particular environments needs to be done with caution.

Climate change is heavily impacting viticulture (Schultz, 2000; Fraga et al., 2012; Hannah et al., 2013) and growers need to adapt to changing climatic conditions in order to continue the production of quality wines at economically sustainable yields (van Leeuwen and Destrac-Irvine, 2017). These adaptations include plant material (Duchêne, 2016), training systems (van Leeuwen et al., 2019), and pest management (Bois et al., 2017). In this context of climate change, it is also critical to better understand current climate, in order to establish a baseline for work on future adaptation.

Extreme weather events, such as frost or heat waves, can impact plant development and cause damage on vine organs (including grape berries) and alter wine quality and typicity. Daily maps of temperature distribution produced in this study can be used to better understand the spatial distribution of these extreme weather events as was done previously for frost event in Champagne and in South Africa (Madelin and Beltrando, 2005; Bonnardot et al., 2012). These maps could be used to define the most sensitive parcels in order to implement adaptations, or to optimize the location of systems like wind turbines for spring frost protection.

In the future, it will be interesting to combine temperature projections under various climate change scenarios (IPCC, 2013) with temperature model developed at the local scale (Le Roux et al., 2017a) in order to improve the accurate of the projections and to help winegrowers to anticipate adaptation.

CONCLUSION

In this study, temperature variability was investigated at a vineyard scale over an area of 19,233 ha within the appellations of Saint-Émilion, Pomerol, and their satellites (Bordeaux, France). Results show a great spatial and temporal variability in temperature. The main factors driving this spatial

temperature distribution, including environmental features and meteorological conditions, are explored. Impact of temperature on vine phenology was investigated by means of the coupling of spatial temperature models with phenology models. Local-scale maps of temperature and corresponding occurrence of phenological stages were created over this area. These maps allow improved adaptation of plant material and training systems to local temperature variability over the area. It is also a useful tool for adaptation of plant material and viticultural practices in the context of climate change.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

LR conducted the study, contributed to data acquisition and statistical analysis, and elaborated the manuscript. TP contributed to data acquisition and temperature database development. SM contributed to the statistical analyses and wrote a part of the manuscript dedicated to statistics. RL contributed to the spatial modeling of temperature indicators and phenological stages. HQ and CL coordinated the study and participated in writing of the manuscript.

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REFERENCES

Alikadic, A., Pertot, I., Eccel, E., Dolci, C., Zarbo, C., Caffarra, A., et al. (2019). The impact of climate change on grapevine phenology and the influence of altitude: a regional study. *Agric. For. Meteorol.* 271, 73–82. doi: 10.1016/j.agrformet.2019.02.030

Allamy, L., Darriet, P., and Pons, A. (2017). Identification and organoleptic contribution of (Z)-1, 5-octadien-3-one to the flavor of *Vitis vinifera* cv. Merlot and Cabernet Sauvignon musts. *J. Agric. Food Chem.* 65, 1915–1923. doi: 10.1021/acs.jafc.6b05293

Baggiolini, M. (1952). Stade repère de la vigne. *Romande Agric. Vitic.* 8, 4–6.

Beltrando, G. (2000). La climatologie: une science géographique. *L'Inf. Géogr.* 64, 241–261. doi: 10.3406/ingeo.2000.2705

Bock, A., Sparks, T., Estrella, N., and Menzel, A. (2011). Changes in the phenology and composition of wine from Franconia, Germany. *Clim. Res.* 50, 69–81. doi: 10.3354/cr01048

Bodin, F., and Morlat, R. (2006). Characterization of Viticultural Terroirs using a Simple Field Model Based on Soil Depth I. Validation of the Water Supply Regime, Phenology and Vine Vigour, in the Anjou Vineyard (France). *Plant Soil* 281, 37–54. doi: 10.1007/s11104-005-3768-0

Bois, B. (2007). *Cartographie Agroclimatique à Méso-échelle: Méthodologie et Application à la Variabilité Spatiale du Climat en Gironde viticole. Conséquences Pour le Développement de la Vigne et la Maturation du Raisin*. Ph.D. thesis, Université de Bordeaux, Bordeaux.

Bois, B., Joly, D., Quénol, H., Pieri, P., Gaudillère, J.-P., Guyon, D., et al. (2018). Temperature-based zoning of the Bordeaux wine region. *OENO One* 52, 291–306. doi: 10.20870/oeno-one.2018.52.4.1580

Bois, B., Zito, S., and Calonnec, A. (2017). Climate vs grapevine pests and diseases worldwide: the first results of a global survey. *OENO One* 51, 133–139. doi: 10.20870/oeno-one.2017.51.2.1780

Bonnardot, V., Carey, V. A., Madelin, M., Cautenet, S., Coetzee, Z., and Quénol, H. (2012). Spatial variability of night temperatures at a fine scale over the Stellenbosch wine district, South Africa. *J. Int. Sci. Vigne Vin* 46, 1–13. doi: 10.20870/oeno-one.2012.46.1.1504

Bonnardot, V., and Cautenet, S. (2009). Mesoscale atmospheric modeling using a high horizontal grid resolution over a complex coastal terrain and a wine region of South Africa. *J. Appl. Meteor. Climatol.* 48, 330–348. doi: 10.1175/2008JAMC1710.1

Bonnefoy, C. (2013). *Observation et Modélisation Spatiale de la Température Dans les Terroirs Viticoles du Val de Loire dans le Contexte du Changement Climatique*. Ph.D. thesis: Université Rennes, Rennes.

Bonnefoy, C., Quénol, H., Bonnardot, V., Barbeau, G., Madelin, M., Planchon, O., et al. (2013). Temporal and spatial analyses of temperature in a French wine-producing area: the Loire Valley. *Int. J. Climatol.* 33, 1849–1862. doi: 10.1002/joc.3552

Cantat, O., Savouret, E., and Bensaïd, A. (2012). "La régionalisation des types de temps en France métropolitaine," in *Actes du XXVème Colloque de l'Association Internationale de Climatologie*, Grenoble, 165–170.

Carrega, P. (1994). *Topoclimatologie et Habitat. Analyse Spatiale Quantitative et Appliquée*. Ph.D. thesis, Université de Nice Sophia-Antipolis, Nice.

Chuine, I., de Cortazar-Atauri, I. G., Kramer, K., and Hänninen, H. (2013). "Plant development models," in *Phenology: An Integrative Environmental Science*, ed. M. D. Schwartz (Dordrecht: Springer), 275–293.

Cocks, C., Féret, E., and Boidron, B. (2014). *Bordeaux et Ses Vins*, 2ème édition Edn, ed. E. Féret (Bordeaux: Université de Bordeaux).

Cohen, S. D., Tarara, J. M., Gambetta, G. A., Matthews, M. A., and Kennedy, J. A. (2012). Impact of diurnal temperature variation on grape berry development, proanthocyanidin accumulation, and the expression of flavonoid pathway genes. *J. Exp. Bot.* 63, 2655–2665. doi: 10.1093/jxb/err449

Coombe, B. G. (1987). Influence of temperature on composition and quality of grapes. *Acta Hortic.* 23–36. doi: 10.17660/ActaHortic.1987.206.1

Cortes, C., and Vapnik, V. (1995). Support-vector networks. *Mach. Learn.* 20, 273–297. doi: 10.1007/BF00994018

Cuccia, C. (2013). *Impacts du Changement Climatique sur la Phénologie du Pinot noir en Bourgogne*. Ph.D. thesis, Université de Borgogne, Dijon.

Dami, I. E., Ennahli, S., and Zhang, Y. (2012). Assessment of winter injury in grape cultivars and pruning strategies following a freezing stress event. *Am. J. Enol. Vitic.* 63, 106–111. doi: 10.5344/ajev.2011.11040

Daniels, A. E., Morrison, J. F., Joyce, L. A., Crookston, N. L., Chen, S.-C., and McNulty, S. G. (2012). *Climate Projections FAQ*. General Technical Report NO. RMRS-GTR-277WWW. Vol. 32. Fort Collins, CO: US Department of Agriculture, 277. doi: 10.2737/RMRS-GTR-277

De Cortázar-Atauri, I., Duchêne, E., Destrac, A., Barbeau, G., de Rességuier, L., Lacombe, T., et al. (2017). Grapevine phenology in France: from past observations to future evolutions in the context of climate change. *OENO One* 51, 115–126. doi: 10.20870/oeno-one.2016.0.0.1622

De Cortázar-Atauri, I. G., Brisson, N., and Gaudillere, J. P. (2009). Performance of several models for predicting budburst date of grapevine (*Vitis vinifera* L.). *Int. J. Biometeorol.* 53, 317–326. doi: 10.1007/s00484-009-0217-4

de Rességuier, L., Le Roux, R., Petitjean, T., Mary, S., Quénol, H., and van Leeuwen, C. (2018). "Variability of climate, water and nitrogen status and its influence on vine phenology and grape composition inside a small winegrowing estate," in *Proceedings of the XIIème International Terroir Congress*, Zaragoza.

Destrac, A., Flutre, T., Renaud, C., Morin, E., Durand, L., Delrot, S., et al. (2015). "The use of Fourier transform infrared spectroscopy in phenotyping berries from the grapevine *Vitis Vinifera* L," in *Proceedings of the XIXth International Giesco Meeting*, Montpellier, 641–645.

Destrac-Irvine, A., Barbeau, G., de Rességuier, L., Dufourcq, T., Dumas, V., Garcia, et al. (2019). Measuring the phenology to more effectively manage the vineyard. *IVES Tech. Rev.* doi: 10.20870/IVES-TR.2019.2586

Douvinet, J., Planchon, O., Cantat, O., Delahaye, D., and Cador, J.-M. (2009). Variabilité spatio-temporelle et dynamique des pluies de forte intensité à l'origine des «Crues Rapides» dans le bassin parisien (France). *Climatologie* 6, 47–72. doi: 10.4267/climatologie.511

Duchêne, E. (2016). How can grapevine genetics contribute to the adaptation to climate change? *OENO One* 50, 113–124. doi: 10.20870/oeno-one.2016.50.3.98

Duchêne, E., Butterlin, G., Dumas, V., and Merdinoglu, D. (2012). Towards the adaptation of grapevine varieties to climate change: QTLs and candidate genes for developmental stages. *Theor. Appl. Genet.* 124, 623–635. doi: 10.1007/s00122-011-1734-1

Duchêne, E., and Schneider, C. (2005). Grapevine and climatic changes: a glance at the situation in Alsace. *Agron. Sustain. Dev.* 25, 93–99. doi: 10.1051/agro:2004057

Eveno, M., de Rességuier, L., van Leeuwen, C., Cantat, O., Quénol, H., and Planchon, O. (2016). "Analyse de la variabilité spatio-temporelle du climat dans le vignoble de Saint Émilion: étude combinée des types de temps et des types de circulation," in *XXIXème colloque de l'AIC: Climat et pollution de l'air*, 255–260.

Falcão, L. D., de Revel, G., Perello, M. C., Moutsiou, A., Zanus, M. C., and Bordignon-Luiz, M. T. (2007). A survey of seasonal temperatures and vineyard altitude influences on 2-Methoxy-3-isobutylpyrazine, C13-Norisoprenoids, and the Sensory Profile of Brazilian Cabernet Sauvignon Wines. *J. Agric. Food Chem.* 55, 3605–3612. doi: 10.1021/jf070185u

Ferguson, J. C., Moyer, M. M., Mills, L. J., Hoogenboom, G., and Keller, M. (2014). Modeling dormant bud cold hardiness and Budbreak in Twenty-Three Vitis Genotypes Reveals Variation by Region of Origin. *Am. J. Enol. Vitic.* 65, 59–71. doi: 10.5344/ajev.2013.13098

Fraga, H., de Cortazar-Atauri, I. G., Malheiro, A. C., and Santos, J. A. (2016). Modelling climate change impacts on viticultural yield, phenology and stress conditions in Europe. *Glob. Change Biol.* 22, 3774–3788. doi: 10.1111/gcb.13382

Fraga, H., Malheiro, A. C., Moutinho-Pereira, J., and Santos, J. A. (2012). An overview of climate change impacts on European viticulture. *Food Energy Secur.* 1, 94–110. doi: 10.1002/fes3.14

Gladstones, J. (1992). *Viticulture and Environment a Study of the Effects of Environment on Grapegrowing and Wine Qualities, with Emphasis on Present and Future areas for growing Winegrapes in Australia*. Adelaide: Winetitles.

Gladstones, J. (2011). *Wine, Terroir and Climate Change*. Kent: Wakefield Press.

Guyot, G. (1997). *Climatologie de L'environnement: De la Plante aux Écosystèmes*. Paris: Masson.

Hannah, L., Roehrdanz, P. R., Ikegami, M., Shepard, A. V., Shaw, M. R., Tabor, G., et al. (2013). Climate change, wine, and conservation. *Proc. Natl. Acad. Sci. U.S.A.* 110, 6907–6912. doi: 10.1073/pnas.1210127110

Hess, P., and Brezowsky, H. (1952). *Katalog der Grosswetterlagen Europas, Ber Dt Wetterdienstes in der US-Zone, Nr* 33, 1–39.

Hess, W. N. (1974). *Weather and Climate Modification*. New York, NY: Wiley.

Huglin, P., and Schneider, C. (1998). *Biologie et Écologie de la Vigne*. Paris: Lavoisier Tec&doc, 370.

IPCC (2013). *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge: Cambridge University Press, 33–118.

Joly, D., Nilsen, L., Fury, R., Elvebakk, A., and Brossard, T. (2003). Temperature interpolation at a large scale: test on a small area in Svalbard. *Int. J. Climatol.* 23, 1637–1654. doi: 10.1002/joc.949

Jones, G. V. (2006). "Climate and terroir: impacts of climate variability and change on wine," in *Fine Wine and Terroir-the Geoscience Perspective*, eds R. W. Macqueen, and L. D. Meinert (St. John's: Geological association of Canada), 1–14.

Jones, G. V. (2018). The climate component of terroir. *Elements* 14, 167–172. doi: 10.2138/gselements.14.3.167

Jones, G. V., Reid, R., and Vilks, A. (2012). "Climate, grapes, and wine: structure and suitability in a variable and changing climate," in *The Geography of Wine: Regions, Terroir and Techniques*, ed. P. H. Dougherty (Dordrecht: Springer), 109–133. doi: 10.1007/978-94-007-0464-0_7

Kliewer, W. M., and Torres, R. E. (1972). Effect of controlled day and night temperatures on grape coloration. *Am. J. Enol. Vitic.* 23, 71–77.

Köppen, W., and Geiger, R. (1954). *Klima der Erde (Climate of the earth) Wall Map*. Gotha: Klett-Perthes.

Kriedemann, P., and Smart, R. (1971). Effects of irradiance, temperature, and leaf water potential on photosynthesis of vine leaves. *Photosynthetica* 5, 6–15.

Le Roux, R. (2017). *Modélisation Climatique à L'échelle des Terroirs Viticoles Dans un Contexte de Changement Climatique*. Ph.D. thesis, Université Rennes, Rennes.

Le Roux, R., de Rességuier, L., Corpetti, T., Jégou, N., Madelin, M., van Leeuwen, C., et al. (2017a). Comparison of two fine scale spatial models for mapping temperatures inside winegrowing areas. *Agric. For. Meteorol.* 247, 159–169. doi: 10.1016/j.agrformet.2017.07.020

Le Roux, R., de Rességuier, L., Katurji, M., Zawar-Reza, P., Sturman, A., van Leeuwen, C., et al. (2017b). Analyse multiscalaire de la variabilité spatiale et temporelle des températures à l'échelle des appellations viticoles de Saint-Émilion, Pomerol et leurs satellites. *Climatologie* 14, 1–17. doi: 10.4267/climatologie.1243

Leys, C., Ley, C., Klein, O., Bernard, P., and Licata, L. (2013). Detecting outliers: do not use standard deviation around the mean, use absolute deviation around the median. *J. Exp. Soc. Psychol.* 49, 764–766. doi: 10.1016/j.jesp.2013.03.013

Madelin, M., and Beltrando, G. (2005). Spatial interpolation-based mapping of the spring frost hazard in the Champagne vineyards. *Meteorol. Appl.* 12, 51–56. doi: 10.1017/S1350482705001568

Madelin, M., Bois, B., and Quénol, H. (2014). "Variabilité topoclimatique et phénologique des terroirs de la montagne de Corton (Bourgogne)," in *Changement Climatique et Terroirs Viticoles*, ed. H. Quénol (Paris: Lavoisier Tec&doc), 215–227.

Marais, J., Van Wyk, C., and Rapp, A. (1992). Effect of sunlight and shade on norisoprenoid levels in maturing Weisser Riesling and Chenin blanc grapes and Weisser Riesling wines. *S. Afr. J. Enol. Vitic.* 13, 23–32.

Menzel, A., Sparks, T. H., Estrella, N., Koch, E., Aasa, A., Ahas, R., et al. (2006). European phenological response to climate change matches the warming pattern. *Glob. Change Biol.* 12, 1969–1976. doi: 10.1111/j.1365-2486.2006.01193.x

Mira, and de Orduña, R. (2010). Climate change associated effects on grape and wine quality and production. *Food Res. Int.* 43, 1844–1855. doi: 10.1016/j.foodres.2010.05.001

Montes, C., Perez Quezada, J. F., Peña Neira, A., and Tonietto, J. (2012). Climatic potential for viticulture in Central Chile. *Aust. J. Grape Wine Res.* 18, 20–28. doi: 10.1111/j.1755-0238.2011.00165.x

Mori, K., Goto-Yamamoto, N., Kitayama, M., and Hashizume, K. (2007). Loss of anthocyanins in red-wine grape under high temperature. *J. Exp. Bot.* 58, 1935–1945. doi: 10.1093/jxb/erm055

Morlat, R., and Bodin, F. (2006). Characterization of viticultural terroirs using a simple field model based on soil depth – II. Validation of the grape yield and berry quality in the Anjou Vineyard (France). *Plant Soil* 281, 55–69. doi: 10.1007/s11104-005-3769-z

Neethling, E., Barbeau, G., Coulon-Leroy, C., and Quénol, H. (2019). Spatial complexity and temporal dynamics in viticulture: a review of climate-driven scales. *Agric. For. Meteorol.* 276-277:107618. doi: 10.1016/j.agrformet.2019.107618

Parker, A., de Cortázar-Atauri, I. G., Chuine, I., Barbeau, G., Bois, B., Boursiquot, J.-M., et al. (2013). Classification of varieties for their timing of flowering and veraison using a modelling approach: a case study for the grapevine species *Vitis*

vinifera L. *Agric. For. Meteorol.* 180, 249–264. doi: 10.1016/j.agrformet.2013.06.005

Parker, A. K., De Cortázar-Atauri, I. G., van Leeuwen, C., and Chuine, I. (2011). General phenological model to characterise the timing of flowering and veraison of *Vitis vinifera* L.: Grapevine flowering and veraison model. *Aust. J. Grape Wine Res.* 17, 206–216. doi: 10.1111/j.1755-0238.2011.00140.x

Parker, A. K., García, de Cortázar-Atauri, I., Gény, L., Spring, J.-L., Destrac, A., et al. (2020). Temperature-based grapevine sugar ripeness modelling for a wide range of *Vitis vinifera* L. cultivars. *Agric. For. Meteorol.* 285–286:107902. doi: 10.1016/j.agrformet.2020.107902

Pinheiro, J. C., and Bates, D. M. (2000). "Linear mixed-effects models: basic concepts and examples," in *Mixed-Effects Models in S and S-Plus*, ed. Springer-Verlag New York, 3–56. doi: 10.1007/978-1-4419-0318-1_1

Planchon, O., Cantat, O., and Quénol, H. (2015). "Types de temps et types de circulation atmosphérique: essai de mise en relation à Angers et Bordeaux (France)," in *Proceedings of the XXVIIIe Colloque de l'Association Internationale de Climatologie*, Liège, 381–386.

Planchon, O., Quénol, H., Dupont, N., and Corgne, S. (2009). Application of the Hess-Brezowsky classification to the identification of weather patterns causing heavy winter rainfall in Brittany (France). *Nat. Hazards Earth Syst. Sci.* 9, 1161–1173. doi: 10.5194/nhess-9-1161-2009

Poling, E. B. (2008). Spring cold injury to winegrapes and protection strategies and methods. *Hortscience* 43, 1652–1662. doi: 10.21273/HORTSCI.43.6.1652

Pons, A., Allamy, L., Schüttler, A., Rauhut, D., Thibon, C., and Darriet, P. (2017). What is the expected impact of climate change on wine aroma compounds and their precursors in grape? *OENO One* 51, 141–146. doi: 10.20870/oeno-one.2017.51.2.1868

Quénol, H. (2014). *Changement Climatique et Terroirs Viticoles*. Paris: Lavoisier Tec&doc.

Quénol, H., Aruani, C., Fourment, M., Trapeteau, L., Grassin, M., Briche, E., et al. (2014). "Changement climatique dans les vignobles d'Amérique du Sud," in *Changement Climatique et Terroirs Viticoles*, ed Lavoisier Tec&doc (Paris: Lavoisier Tec&doc), 291–315.

Quénol, H., and Bonnardot, V. (2014). A multi-scale climatic analysis of viticultural terroirs in the context of climate change: the "teradclim" project. *J. Int. Sci. Vigne Vin* 47, 23–32.

Quénol, H., Garcia, de Cortazar Atauri, I., Bois, B., Sturman, A., Bonnardot, V., et al. (2017). Which climatic modeling to assess climate change impacts on vineyards? *OENO One* 51, 91–97. doi: 10.20870/oeno-one.2016.0.0.1869

Quénol, H., Monteiro, A., Beltrando, G., and Maciel, A. (2004). Mesures climatiques aux échelles fines (météorologiques et agronomiques) et variabilité spatiale du gel printanier dans le vignoble de Vinho Verde (Portugal). *Norois* 117–132. doi: 10.4000/norois.826

R Core Team (2016). *R: A Language and Environment for Statistical Computing*. Vienna: R Development Core Team.

Reynolds, A. G., Senchuk, I. V., Reest, C., van der, and Savigny, C. (2007). Use of GPS and GIS for elucidation of the basis for terroir: spatial variation in an Ontario Riesling Vineyard. *Am. J. Enol. Vitic.* 58, 145–162.

Rhoades, A. M., Huang, X., Ullrich, P. A., and Zarzycki, C. M. (2015). Characterizing Sierra Nevada Snowpack using variable-resolution CESM. *J. Appl. Meteor. Climatol.* 55, 173–196. doi: 10.1175/JAMC-D-15-0156.1

Rousseeuw, P. J., and Croux, C. (1993). Alternatives to the median absolute deviation. *J. Am. Stat. Assoc.* 88, 1273–1283. doi: 10.1080/01621459.1993.10476408

Scherrer, D., and Körner, C. (2011). Topographically controlled thermal-habitat differentiation buffers alpine plant diversity against climate warming. *J. Biogeogr.* 38, 406–416. doi: 10.1111/j.1365-2699.2010.02407.x

Schultz, H. R. (2000). Climate change and viticulture: a European perspective on climatology, carbon dioxide and UV-B effects. *Aust. J. Grape Wine Res.* 6, 2–12. doi: 10.1111/j.1755-0238.2000.tb00156.x

Sgubin, G., Swingedouw, D., Dayon, G., García, de Cortázar-Atauri, I., Ollat, N., et al. (2018). The risk of tardive frost damage in French vineyards in a changing climate. *Agric. For. Meteorol.* 250–251, 226–242. doi: 10.1016/j.agrformet.2017.12.253

Spayd, S. E., Tarara, J. M., Mee, D. L., and Ferguson, J. C. (2002). Separation of sunlight and temperature effects on the composition of *Vitis vinifera* cv. Merlot Berries. *Am. J. Enol. Vitic.* 53, 171–182.

Stahl, K., Moore, R. D., Floyer, J. A., Asplin, M. G., and McKendry, I. G. (2006). Comparison of approaches for spatial interpolation of daily air temperature in a large region with complex topography and highly variable station density. *Agric. For. Meteorol.* 139, 224–236. doi: 10.1016/j.agrformet.2006.07.004

Sturman, A., Zawar-Reza, P., Soltanzadeh, I., Katurji, M., Bonnardot, V., Parker, A. K., et al. (2017). The application of high-resolution atmospheric modelling to weather and climate variability in vineyard regions. *OENO One* 51:99. doi: 10.20870/oeno-one.2016.0.0.1538

Swinchatt, J. P., Howell, D. G., and MacDonald, S. L. (2018). The scale dependence of wine and Terroir: examples from coastal California and the Napa Valley (USA). *Elements* 14, 179–184. doi: 10.2138/gselements.14.3.179

Tarara, J. M., Lee, J., Spayd, S. E., and Scagel, C. F. (2008). Berry Temperature and Solar Radiation Alter Acylation, Proportion, and Concentration of Anthocyanin in Merlot Grapes. *Am. J. Enol. Vitic.* 59, 235–247.

Tomasi, D., Jones, G. V., Giust, M., Lovat, L., and Gaiotti, F. (2011). Grapevine Phenology and Climate Change: relationships and Trends in the Veneto Region of Italy for 1964–2009. *Am. J. Enol. Vitic.* 62, 329–339. doi: 10.5344/ajev.2011.10108

Tonietto, J., and Carbonneau, A. (2004). A multicriteria climatic classification system for grape-growing regions worldwide. *Agric. For. Meteorol.* 124, 81–97. doi: 10.1016/j.agrformet.2003.06.001

Ubalde, J. M., Sort, X., Zayas, A., and Poch, R. M. (2010). Effects of soil and climatic conditions on grape ripening and wine quality of cabernet sauvignon. *J. Wine Res.* 21, 1–17. doi: 10.1080/09571264.2010.495851

van Leeuwen, C., and de Rességuier, L. (2018). Major soil-related factors in terroir expression and Vineyard Siting. *Elements* 14, 159–165. doi: 10.2138/gselements.14.3.159

van Leeuwen, C., and Destrac-Irvine, A. (2017). Modified grape composition under climate change conditions requires adaptations in the vineyard. *OENO One* 51:147. doi: 10.20870/oeno-one.2016.0.0.1647

van Leeuwen, C., Destrac-Irvine, A., Dubernet, M., Duchêne, E., Gowdy, M., Marguerit, E., et al. (2019). An update on the impact of climate change in viticulture and potential adaptations. *Agronomy* 9:514. doi: 10.3390/agronomy9090514

van Leeuwen, C., Friant, P., Choné, X., Tregoat, O., Koundouras, S., and Dubourdieu, D. (2004). Influence of climate, soil and cultivar on Terroir. *Am. J. Enol. Vitic.* 55, 207–217.

van Leeuwen, C., and Seguin, G. (2006). The concept of terroir in viticulture. *J. Wine Res.* 17, 1–10. doi: 10.1080/09571260600633135

van Leeuwen, C., van, Baudet, D., Duteau, J., Seguin, G., and Wilbert, J. (1989). Les sols viticoles et leur répartition a Saint-Emilion, Pomerol et quelques autres communes du Libournais. *J. Int. Sci. Vigne. Vin.* 23, 131–150. doi: 10.20870/oeno-one.1989.23.3.1243

Vaudour, E., Costantini, E., Jones, G. V., and Mocali, S. (2015). An overview of the recent approaches to terroir functional modelling, footprinting and zoning. *Soil* 1, 287–312. doi: 10.5194/soil-1-287-2015

Wang, E., and Engel, T. (1998). Simulation of phenological development of wheat crops. *Agric. Syst.* 58, 1–24. doi: 10.1016/S0308-521X(98)00028-6

Winkler, A. J. (1974). *General Viticulture*. Berkeley, CA: University of California Press.

Wu, T., and Li, Y. (2013). Spatial interpolation of temperature in the United States using residual kriging. *Appl. Geogr.* 44, 112–120. doi: 10.1016/j.apgeog.2013.07.012

Xu, Y., Castel, T., Richard, Y., Cuccia, C., and Bois, B. (2012). Burgundy regional climate change and its potential impact on grapevines. *Clim. Dyn.* 39, 1613–1626. doi: 10.1007/s00382-011-1284-x

Zabadal, T. J., Dami, I. E., Goffinet, M. C., Martinson, T. E., and Chien, M. L. (2007). *Winter Injury to Grapevines and Methods of Protection*. East Lansing, MI: Michigan State University Extension.

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Microbial Functional Diversity in Vineyard Soils: Sulfur Metabolism and Links With Grapevine Plants and Wine Quality

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The quality of the vineyard soils has a direct impact on grapes and wine quality and represents a key component of the "*Terroir concept*." However, information on the impact of soil microbiota on grapevine plants and wine quality are generally lacking. In fact, over the last few years most of the attempts made to correlate soil microbial communities and wine quality were limited by overlooking both the functional traits of soil microbiota and the spatial variability of vineyards soils. In this work, we used a functional gene microarray approach (GeoChip) and soil enzymatic analyses to assess the soil microbial community functional potential related to the different wine quality. In order to minimize the soil variability, this work was conducted at a "within-vineyard" scale, comparing two similar soils (BRO11 and BRO12) previously identified with respect to pedological and hydrological properties within a single vineyard in Central Tuscany and that yielded highly contrasting wine quality upon cultivation of the same Sangiovese cultivar (BRO12 exhibited the higher quality). Our results showed an enrichment of Actinobacteria in BRO12, whereas Alfa- and Gamma-Proteobacteria were more abundant in BRO11, where an enrichment of bacteria involved in N fixation and denitrification occurred. Overall, the GeoChip output revealed a greater biological activity in BRO11 but a significant enrichment of sulfur-oxidation genes in BRO12 compared to BRO11 soil, where a higher level of arylsulfatase activity was also detected. Moreover, the low content of sulfates and available nitrogen found in BRO12 suggested that the reduced availability of sulfates for vine plants might limit the reduced glutathione (GSH) synthesis, which plays an important role in aroma protection in musts and wines. In conclusion, in addition to nitrogen availability, we propose that soil microbial sulfur metabolism may also play a key role in shaping plant physiology, grapes and wine quality. Overall, these results support the existence of a "microbial functional terroir" effect as a determining factor in vineyard-scale variation among wine grapes.

Keywords: microbial terroir, sulfur cycling, wine quality, soil biodiversity, GeoChip

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INTRODUCTION

The relationship between soil and crop quality has been studied intensively over the past several decades, especially with respect to grapes used in wine production (Seguin, 1986; Vaudour, 2002; Deloire et al., 2005). The quality of wine is largely determined by grapes but a number of factors, such as grapes variety and vine rootstock, water availability, climate, soil properties, and viticultural-oenological practices directly affect grape quality at harvest (OIV, 2010). The interactions among such factors over time determine the "*Terroir concept*," an interactive ecosystem which define a specific vineyard site with unique features that give wine grapes their distinctive character (van Leeuwen and Seguin, 2006). Although many studies have demonstrated that soil chemical, physical and hydrological features can strongly affect wine peculiarities (van Leeuwen et al., 2004; Vaudour et al., 2015), the potential contribution of soil microbiota has until recently been overlooked (Bokulich et al., 2014; Gilbert et al., 2014; Knight et al., 2015; Garofalo et al., 2016; Miura et al., 2017). In fact, whereas the uniqueness of the microbiota present typically on the skins of the fruit, and how this influences the compounds produced during fermentation, is a well-accepted concept (Barata et al., 2012; Felder et al., 2012; Bokulich et al., 2014, 2016; Capozzi et al., 2015; Knight et al., 2015; Jara et al., 2016; Mezzasalma et al., 2017), the uniqueness of the microbial community structure found in the soil and/or associated to various plant parts which influence the flavor, color, and quality of the fruit and wine is not clearly established (Gilbert et al., 2014). In the last few years several authors tried to support the "microbial terroir" concept suggesting that soil microbiome may affect wine quality (Burns et al., 2015; Zarraonaindia et al., 2015; Bokulich et al., 2016; Belda et al., 2017; Vadakattu et al., 2019). However, the results generally highlighted distinct microbial communities correlated to different vineyards but without finding any direct effect on vine growth, fruit properties, or wine quality. In fact, as the authors indicate, "correlation is not causation" and more research is needed to link specific microbial functions to one or more sensory features of wines. Moreover, most of such studies have been conducted on different vineyards or soils located in different areas, thus providing severe limitations for the assignment of any soil microbial taxa/groups associated to a specific vine plant. In fact, it is well-known that microbial community structure and biogeography change with environmental variations and are primarily controlled by edaphic features (Fierer and Jackson, 2006; Kuramae et al., 2012; Burns et al., 2015; Li et al., 2019). Thus, in order to assess the role of soil microbial diversity and functions in determining grape and wine quality, it is essential to minimize the environmental variability and conduct the research at a within-vineyard scale, in the same soil or among similar soils.

Thus, in this study we selected two experimental plots within the same vineyard in Chianti municipality (Tuscany, Italy), corresponding to subareas with highly similar soils, called BRO11, and BRO12. Remarkably, although their similarity, BRO11 and BRO12 soils gave rise to grape and wines that differ in their quality (Costantini et al., 2013).

This experimental field thus provided a means to examine the microbial community properties of two highly similar soils giving rise to wine grapes of disparate quality, thereby potentially providing a means to examine how soil microbial community structure and functions are related to grapevine performance and wine quality. Here, we hypothesized that soil microbial functions are different in these two plots that might exert a key role in shaping the grapevine performance and wine quality. To test this hypothesis, we (i) determined the soil properties, compared the (ii) microbial functional diversity and major metabolic pathways using microbial function microarray (Geochip 3.0), and (iii) soil enzyme activity involved in C, N, and P cycles in plots BRO11 and BRO12. Results are discussed with respect to the potential factors effecting microbial communities in these soils, potential links between microbial community properties and final grape and wine quality and the general notion of a *microbial terroir* concept.

MATERIALS AND METHODS

Description of the Study Area

The selected vineyard is located in the Brolio farm (lat: 43.384°, long: 11.436°), which belongs to the Barone Ricasoli estate, one of the most important wineries of the Chianti Classico wine-area, in Gaiole in Chianti municipality (Tuscany region, Italy). The vineyard was established in 2000, after weak slope re-shaping and deep plowing to a depth of 0.8–1.0 m. The grape cultivar was *Sangiovese*, which is the main variety of Chianti Classico and other red wines of central Italy. Plant density was 6,250 plants/ha (2.0 × 0.8 m) and the rootstock was 420A.

This vineyard has been the subject of previous research projects aimed to determine the relationships between soil and wine quality, in which a total of 3 + 3 years of mapping and monitoring activities on grapevines and soils have been undertaken. Some results of research activities relating to water and nitrogen nutrition were already published by Costantini et al. (2013), whereas other results on geophysical investigation were published by Martini et al. (2013) and Braschi et al. (2018). The depositional sequence started in the bottom by clayey deposits, some tens of meters thick, characterized by small lignite lenses and gypsum crystals, overlying by sandy-gravelly deposits of beach environment, for an average thickness, determined by geophysical analysis (Martini et al., 2013), of about 10 meters. The erosion during Quaternary period brought the clayey deposits on the surface only along valley and in the lower part of the slope. In this vineyard, about 70% of the total surface is characterized by sandy-gravelly marine deposits, whereas the lower part of the slope (<310 m a.s.l.) shows clayey deposits on the surface or buried by shallow (20–30 cm) deposits of sands, recently sedimented for colluviation from the slope.

The two plots selected for this study, BRO11 and BRO12, were about 80 m apart on a single vineyard row in the same soil typological unit (**Figure 1** and **Figure S1**), and developed on marine sands and conglomerates dating back to 4.5 million years B.P. (Early Pliocene period). Both soils have sandy loam texture, moderate calcium carbonate content (60–150 $g \cdot kg^{-1}$), subalkaline pH (8.2–8.4), and low organic carbon (2.3–6.5 $g \cdot kg^{-1}$). The soil moisture monitoring of the 0–70 cm evidenced a very similar water availability among the two soils (Costantini et al., 2013). BRO12 has little higher content of sand (about

FIGURE 1 | Pictures of the studied vineyard (from North to Southward) and soil profiles of BRO11 and BRO12 plots.

+10%) and coarse fragments (about +10%), as well as slightly lower organic carbon (about −4 g·kg^{-1}) and available water capacity (AWC, −10 mm·m^{-1}).

Despite the high similarity of the two soils, the grapevines response in the two plots were different, as reported in the previous paper (Costantini et al., 2013). Briefly, the ratio between the carbon isotopes $^{13}C/^{12}C$ (δ13C) found in the wines demonstrated higher grapevine water stress during summer in BRO11 than in BRO12. However, BRO12 produced lower grape yield/plant during experimental years (2008/'09/'10), which on average was about 1 kg/plant vs. 1.6 kg/plant of BRO11. On the other hand, the grapes of BRO12 showed a small increase of the average sugar content (23.2 °Brix, instead 22.2 °Brix of BRO11), extractable polyphenols (1,883 vs. 1,638 mg·l^{-1}) and significantly ($p < 0.05$) higher total anthocyanins (1,500 vs. 1,316 mg ·l^{-1}, data available only for 2008 and 2009). The overall wine quality was determined by means of the "Vine Performance of Sangiovese" (VPS) index (Bucelli et al., 2010), which showed a higher VPS value (VPS = 81) in BRO12 than in BRO11 (VPS = 62) over the three vintages.

Pedological Survey and Soil Sampling

In order to assess the soil spatial homogeneity across the vineyard, the experimental field was surveyed in greater detail by the use of soil proximal sensors, namely electromagnetic induction sensor (EM38- Mk2, Geonics Ltd., Canada) and gamma-ray spectroscopy (The Mole, Soil Company, The Netherlands), able to provide a cheap, non-invasive, and rapid mapping of the soil apparent electric conductivity (ECa) and soil stoniness and mineralogy, respectively (Priori et al., 2013, 2014). The maps obtained by proximal soil sensing defined the homogeneity of the study area and allowed to select the sampling points within two homogeneous plots. At the harvest, three sub-plots (replicates) of the two soils were then laid out along the same vineyard row, at about 5 m from each other. Five soil sub-samples were collected at the same distance from the plants (about 50 cm across the row), at 0–30 cm depth and accurately mixed together in a unique sample for each sub-plot (about 1 kg each). Soil samples were air-dried at room temperature and sieved at 2 mm before being stored at −30°C for the subsequent GeoChip and enzymatic analysis.

In the same vineyard row, two soil profiles (BRO11 and BRO12) were dug until a depth of about 1.3 m, and described by following the international guidelines for soil description (Jahn et al., 2006). For laboratory physical and chemical analysis, each soil horizon of the soil profiles was collected, air-dried and 2.0 mm sieved; the resulting sample was then stored at room temperature before being analyzed.

Soil Chemical and Physical Analyses

Soil texture was determined by a X-ray/sedimentation throughout a Micromeritics Sedigraph III analyser, according to Andrenelli et al. (2013). Total soil organic carbon (SOC) and nitrogen (TN) were determined by dry combustion using a ThermoFlash 2000 CN soil analyzer, after removing the carbonates with HCl 10%. The total equivalent $CaCO_3$ was calculated as the difference of total C between the untreated soil (mineral C + organic C) and the HCl-treated soil (organic C). The active lime was determined using the Drouineau method in accordance with Loeppert and Suarez (1996). The soil pH was measured potentiometrically in a 1:2.5 soil–water suspension. The soil cation exchange capacity (CEC) and the exchange bases were determined with the $BaCl_2$-triethanolamine (pH 8.2) method, whereas the amount of Ca, Mg, K, and Na in the extracts were quantified by flame atomic absorption spectrometry (Agilent SpectrAA 220FS spectrometer) (Gessa and Ciavatta, 2000). Finally, the total SO_4-S was determined by turbidimetric assay according with the standard method for water-soluble sulfate in soil proposed by the ASTM International (2015).

DNA Extraction, Purification, and Whole Genome Amplification

The total DNA was extracted from a 5 g soil sample using a procedure including freezing, grinding and thawing samples in liquid nitrogen (3 times), and treatment with sodium dodecyl sulfate (SDS) and cetyl-trimethylammonium bromide (CTAB)

for cell lysis and DNA extraction, respectively, in accordance with Richard et al. (2001). The DNA was then purified with the Promega Wizard DNA Clean-Up System (Madison, WI, USA), in accordance with the manufacturer's instruction. Then, the DNA quality was assessed by gel electrophoresis (1% agarose), stained with 0.5 μg/mL ethidium bromide solution. Both quantity and quality of the DNA samples were carefully checked by means of a Nanodrop Lite spectrophotometer (Thermo Fisher Scientific). Only DNA samples with concentration exceeding 8.5 ng μl^{-1} and 260/280 ratio between 1.5 and 1.9 were considered viable for subsequent processing, according to van Nostrand et al. (2010a). In order to reach the amount and the quality of DNA requested for the Geochip analysis, a whole genome amplification was carried out on 100 ng DNA using the Templiphi Amplification kit (GE Healthcare Life Sciences, Piscataway, NJ) with the following modifications: 0.1 μmol/L spermidine and 260 ng/μL single stranded DNA binding protein (Single Stranded DNA Binding Protein, SSB) were added to enhance the amplification efficiency and representativeness (van Nostrand et al., 2010b). The final DNA samples were then stored at −80°C until further analysis.

GeoChip™ Hybridization and Analysis

GeoChip™ 3.0 was used for DNA hybridization. It is a functional gene array containing approximately 28,000 DNA probes targeting 292 functional gene families involved in most of the main soil processes, such as: carbon (C), nitrogen (N), phosphate (P), and sulfur (S) cycling, energy metabolism, antibiotic resistance, metal resistance, and organic contaminant degradation. Moreover, the DNA gyrase (gyrB) gene is included in the GeoChip v.3.0, and it is useful for comparing microbial community structure across a broad range of taxonomic levels (He et al., 2010a; Lu et al., 2012). Hybridization and subsequent analysis of the DNA samples were carried out by the Institute for Environmental Genomics (IEG) at the University of Oklahoma (USA), as previously described (van Nostrand et al., 2010a). Briefly, 2.5 μg DNA was labeled for fluorescence with Cy5 (GE Healthcare) and the entire processing pipeline was conducted as previously described (Zhang et al., 2006). After hybridization, microarrays were scanned (NimbleGen MS200 Microarray Scanner, Madison, WI, USA) and the signal intensities were quantified using the customized pipeline at IEG (http://ieg.ou.edu/microarray), as described previously (He et al., 2010b). Spots with a signal to-noise ratio (SNR) <2.0 were removed, and a signal intensity 2,000 was considered as threshold value. The raw signal was then log-transformed and normalized, and its relative abundance was calculated in each sample using internal and external standards (Li et al., 2014). Alpha-diversity was determined using Shannon–Wiener index (H), Simpson index (1/D), and Simpson evenness (E). The shared (overlapped) genes between all samples were calculated by dividing the number of overlapping genes by the total genes present in both samples. The BRO11/BRO12 ratio was calculated by dividing the difference occurring among BRO11 and BRO12 unique values and their sum. The entire dataset was submitted to the public repository "Gene Expression Omnibus (GEO) of the NCBI and it is available at the following link: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE146289."

Soil Enzyme Assays

The following enzymatic activities related to C, N, P, S cycles were determined on BRO11 and BRO12 soils: β-glucosidase (gluc), leucine aminopeptidase (leu), pyrophosphatase (pyro), alkaline phosphomonoesterase (alkP), phosphodiesterase (bis-P), and arylsulphatase (aryS). An heteromolecular exchange procedure was used to desorb enzymes from soil, and the subsequent extraction was carried out through a bead-beating step, in accordance with Cowie et al. (2013). Briefly, 400 mg of moist soil plus 1.4 mL of 30 gL^{-1} lysozyme solution as desorbent, plus 0.4 mL glass beads (<100 μm) and 0.4 mL ceramic beads were put in a 2-mL Eppendorf tube. Tubes were bead-beating for 180 s at 30 strokes s^{-1} (Retsch MM400 beating mill) and followed by centrifugation at 20,000 g for 5 min. Afterwards, enzyme activities were quantified by fluorometric assay and results were expressed as nanomoles of fluorophore per gram of soil (dry basis) per hour. Fluorophore was 4-methyl-umbelliferone for β-glucosidase, phosphodiesterase, pyrophosphatase, alkaline phosphomonoesterase, and arylsulphatase, whereas 7-amino-4-methyl coumarine was used for leucine aminopeptidase. The double-stranded DNA (dsDNA) was determined as a measure of soil microbial biomass, as previously reported (Fornasier et al., 2014). The procedure was the same as for enzymes, but the extraction buffer was 0.12 M, pH 7.8 Na-phosphate buffer and bead-beating lasted 120 s. The dsDNA was then quantified by fluorimetry on microplate, without any further purification, using PicoGreen (Life Technologies). The assay was performed according to the manufacturer's instructions, and results were recorded by a microplate reader (Synergy HT, Bio-Tek). All the measurements were done in duplicate.

Statistical Analyses

The overall GeoChip 3.0 data were analyzed and processed at the Institute for Environmental Genomics, University of Oklahoma (http://ieg.ou.edu/). Preprocessed data were then used for further analysis. Functional gene diversity was calculated using Simpson's 1/D, Shannon-Weiner's H′, and evenness. Multivariate detrended correspondence analysis (DCA) of the GeoChip data was used for comparing the different functional gene communities (Zhou et al., 2008). Hierarchical cluster analysis was carried out with Gene Cluster (v. 3.0) and visualized with TreeView on group genes on the basis of the expression pattern (Eisen et al., 1998). Only probes detected in at least 2 out of 3 replicates of each soil sample were considered as positive, regardless the intensity of the signal, and analyzed as binary (0/1) data. Then, the total abundance of each gene category (calculated as the sum of the detected genes for the gene category or family) was used for ANOVA analysis and for determining the BRO11/BRO12 ratio, which was calculated according to the following formula: BRO11/BRO12 ratio = (N11–N12)/(N11+N12) where N11 and N12 are the number of the unique genes detected in BRO11 and BRO12, respectively. SIMPER dissimilarity index was also calculated for the main relevant gene groups. It gives the average percent contribution of the different taxa/genes to the dissimilarity among samples in a Bray-Curtis dissimilarity matrix. Analysis of variance (one-way

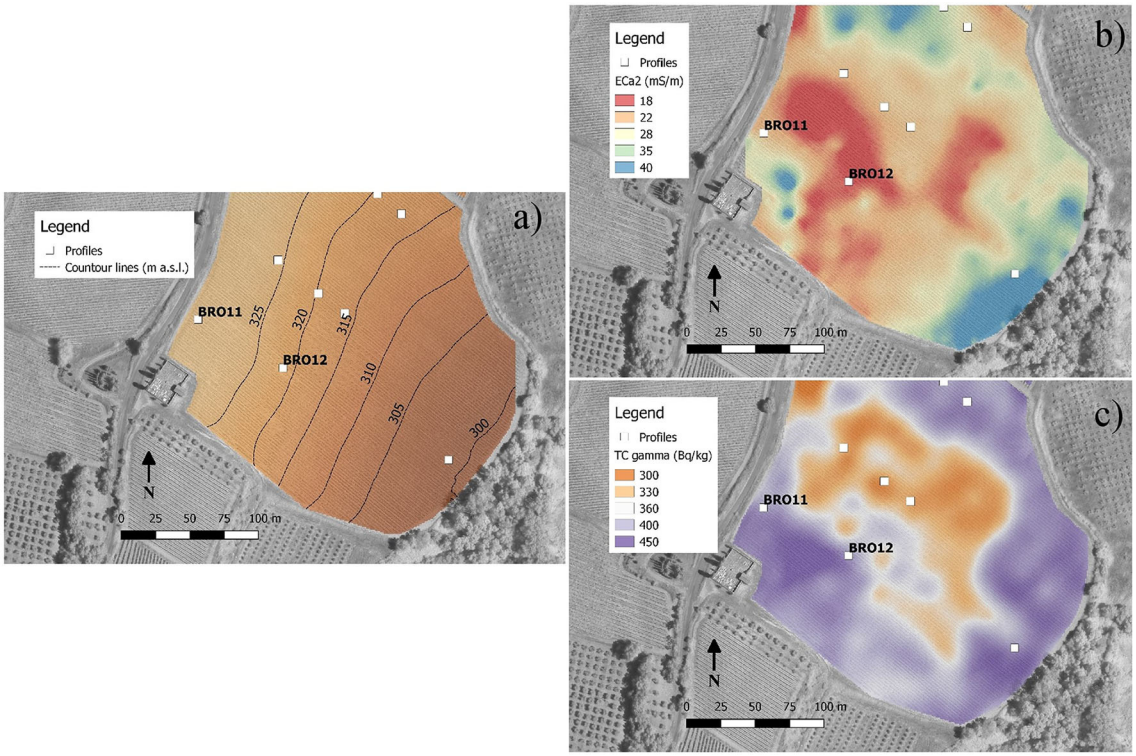


FIGURE 2 | Maps obtained by soil proximal sensing. **(a)** Contour lines of elevation (m a.s.l.); **(b)** apparent electrical conductivity for a soil depth of about 0–150 cm (ECa2) measured by EM38-Mk2 and interpolated by ordinary kriging; **(c)** total counts of gamma-rays emitted from the topsoil (about 0–30 cm).

ANOVA) was used to assess the differences among soil chemical-physical properties (Tukey's test), the functional microbial communities detected by both Geochip and the enzymatic assays. A significance value of $p < 0.05$ was adopted for all comparisons to estimate the statistical difference between the two sites. All the statistical analyses were performed by PAST software v.3.26 (Hammer et al., 2001).

RESULTS

Soil Pedological Survey and Chemical-Physical Analysis

The maps obtained by the interpolation of EM38-MK2 proximal sensor (ECa_1 0–75 cm, map not reported; ECa_2 0–150 cm, **Figure 2**) showed very similar apparent electrical conductivity between BRO11 (ECa_2 = 22 mS/m) and BRO12 (ECa_2 =18 mS/m). These values were coherent with the slight difference in clay content between the sites (**Table 1**). The results of gamma-ray spectroscopy, summarized in **Figure 2** with map of gamma-ray total counts (TC), confirmed the homogeneity of the topsoil in terms of mineralogy and texture within the area between BRO11 and BRO12 with values between 400 and 420 $Bq{\cdot}kg^{-1}$. The chemical-physical analysis of the soil profiles showed an overall higher fertility in BRO11, in terms of SOC, total nitrogen, CEC, Ca, K, Mg, and total sulfates (**Table 1** and **Table S1**).

Overview of the Detected Gene Diversity

The examined samples showed a different number of total detected genes, ranging from 16,688 (BRO12c) to 20,732 (BRO11b) (**Table S2**), showing unique and overlapping genes. In general, soils from BRO11 revealed an average gene number about 12% higher than in BRO12 (**Figure 3A**).

DCA of all detected genes categories showed that the samples from the BRO11 and BRO12 soils were clearly separated along axis 1 (**Figure 3B**). Moreover, BRO11 samples exhibited a high variability along axis 2. Such variability is likely related to the differences occurring among the different gene categories. Moreover, BRO11 and BRO12 samples exhibited different microbial community structure, as shown by the results of both unique and shared (overlapped) genes determined by the phylogenetic marker gyrB (**Table 2**). Thus, to better define the microorganisms involved in soil carbon and nitrogen cycling as well as other key soil processes, selected gene groups were further analyzed.

Phylogenetic Structure of Soil Microbial Communities

The gyrB-based phylogenetic results showed the Proteobacteria phyla as the dominant group, accounting for over 56.6% (262 out of 463) of all the detected genes (**Table 2**), followed by Actinobacteria (14.7%) and Firmicutes (9.3%). Genes from Cyanobacteria (2.8%), Archaea (2.2%), Bacteroides (2.2%), Chlorobi (1.7%), and other (10.5%) were also detected. As

TABLE 1 | Main soil physical and chemical properties.

Site	Horizon	Sand	Clay	pH	$CaCO_3$		SOC	TN	Exchangable complex				SO_4^{2-}
					Tot.	Active			CEC	Ca	K	Mg	
		dag·kg^{-1}			dag·kg^{-1}		g·kg^{-1}		meq·100 g^{-1}				ppm
BRO11	Ap	52^{a}	20^{a}	8.1^{a}	15.6^{a}	3.0^{a}	6.5^{a}	0.7^{a}	10.7^{a}	9.8^{a}	0.3^{a}	0.5^{a}	7.3^{a}
	Bw	60^{b}	17^{a}	8.4^{a}	17.3^{a}	3.3^{a}	2.8^{b}	0.4^{b}	9.8^{a}	9.1^{a}	0.2^{a}	0.4^{a}	8.3^{b}
BRO12	Ap	72^{c}	10^{b}	8.4^{a}	6.4^{b}	1.1^{b}	1.1^{c}	0.2^{c}	7.7^{b}	7.3^{b}	0.1^{b}	0.3^{ab}	6.2^{c}
	Bw	70^{c}	12^{b}	7.8^{b}	5.5^{b}	1.2^{b}	1.2^{c}	0.2^{c}	7.9^{b}	7.5^{b}	0.1^{b}	0.2^{b}	8.1^{b}

For each horizon, sand and clay content, pH, carbonates, soil organic carbon (SOC), total nitrogen (TN), exchangeable complexes (CEC, Ca, K, Mg), and total sulfates (SO_4^{2-}) were determined. Different letters indicate significant differences between topsoil (Ap) and subsoil (Bw) at each site ($p < 0.05$, t-test).

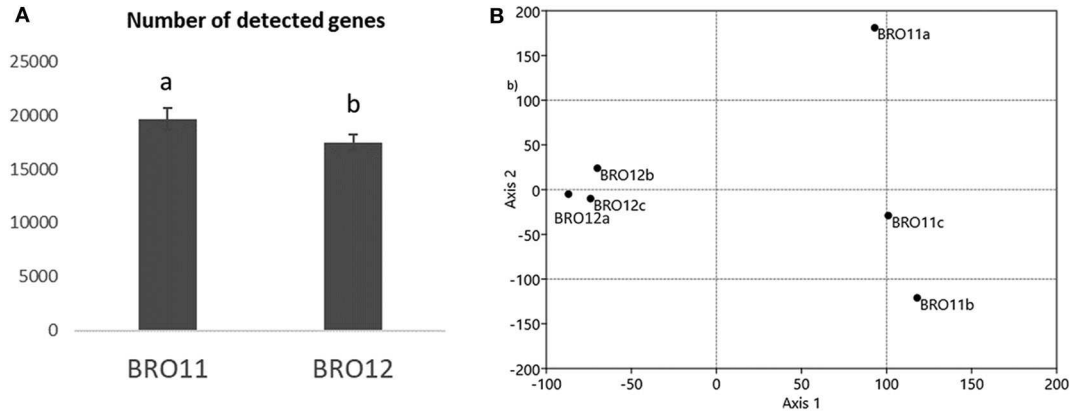


FIGURE 3 | (A) Number of total detected genes by GeoChip analysis. Different letters indicates significant differences of the variable means between the BRO11 and BRO12 soil samples ($p < 0.05$); **(B)** detrended corresponding analysis (DCA) of the overall genes detected by GeoChip 3.0.

shown in **Table 2**, Epsilon- and Beta-Proteobacteria had the most overlapped genes (100 and 93.3%, respectively), while Alpha- and Gamma-Proteobacteria had the fewest values (80.2 and 80%, respectively). In fact, the abundance of Alpha- and Gamma-Proteobacteria was significantly higher in BRO11 than in BRO12 (+22.4%, $p < 0.05$ and +18.2%, $p < 0.001$, respectively), as well as Firmicutes (+7.7%, $p < 0.05$). Conversely, Actinobacteria showed a significant enrichment in BRO12 compared to BRO11 (+4.9%, $p < 0.05$). Moreover, considering the overall abundance of each category, the Simper dissimilarity values indicated Alfa-Proteobacteria (31.7%), Gamma-Proteobacteria (20.7%), and Firmicutes (11.7%) as the highest diverse samples which showed the highest contribution to the overall bacterial diversity between BRO11 and BRO12 sites. Similar results were obtained by calculating the BRO11/BRO12 ratio on the unique genes, except for Alpha-Proteobacteria which exhibited the highest value (0.92). However, as the total number of the genes belonging to the different phylogenetic groups may vary considerably, the contribution of the unique and overlapping genes of BRO11 and BRO12 to the Simper dissimilarity index in all detected genes might be different. For example, the value of the BRO11/BRO12 ratio for Actinobacteria (0.27) is quite lower than for Alpha-Proteobacteria (0.92), indicating that the phylogenetic diversity within Alpha-Proteobacteria bacterial group may be higher than in Actinobacteria, despite the Simper dissimilarity values.

As expected, in general BRO11 showed a higher number of unique genes than BRO12. Interestingly, BRO12 soil showed a higher number of detected genes retrieved from Actinobacteria compared to BRO11 (7 vs. 4), including organisms such as *Micrococcus luteus NCTC 2665*, *Mycetocola lacteus*, *Saccharopolyspora erythraea NRRL 2338*, *Rhodococcus zopfii*, *Gordonia bronchialis,* and *Rubrobacter xylanophilus DSM 9941*.

On the other hand, BRO11 exhibited a higher number of unique genes belonging to Alpha-Proteobacteria (most of which belonging to Rhodobacter, Rhodospirillum, Bradyrhizobium, Jannaschia, Roseobacter, and Methylobacterium genera) and Gamma-Proteobacteria (most of which belonging to Shewanella, Alcanivorax, Enterobacter, and Pseudomonas). Remarkably, among Gamma-Proteobacteria, BRO12 showed higher presence of *Acidithiobacillus ferrooxidans* ATCC 23270 than BRO11.

Interestingly, among Firmicutes an enrichment in *Sulfobacillus thermosulfidooxidans* sp. and *Anoxybacillus flavithermus* WK1 was detected in BRO11 and BRO12, respectively.

Analysis of the Functional Gene Categories

The number of total detected functional genes was 24,676. Among these, 2,164 were involved in C degradation, 537 in C fixation, 37 in methane oxidation, 35 in methane production,

TABLE 2 | Total microbial taxonomy (gyrB) detected in BRO11 and BRO12 soils.

Category	Unique BRO12	Unique BRO11	Shared	Tot	Shared %	Simper %	BRO11/ BRO12 ratio	*P*-value
Actinobacteria	7	4	57	68	83.8	9.5	0.27	0.012*
Alpha-Proteobacteria	1	23	97	121	80.2	31.7	0.92	0.013*
Beta-Proteobacteria	2	1	42	45	93.3	5.1	0.33	0.514
Delta-Proteobacteria	0	2	12	14	85.7	1.9	1.00	0.349
Gamma-Proteobacteria	2	14	64	80	80.0	20.7	0.75	0.001**
Epsilon-Proteobacteria	0	0	2	2	100.0	0.5	0.00	0.373
Archaea	1	0	9	10	90.0	1.8	0.00	0.519
Bacteroides	1	1	8	10	80.0	1.2	0.00	0.643
Firmicutes	1	4	38	43	88.4	11.7	0.60	0.031*
Cyanobacteria	0	1	12	13	92.3	5.3	1.00	0.024*
Chlorobi	0	1	7	8	87.5	1.9	1.00	0.251
Other	2	2	45	49	91.8	8.5	0.00	0.018*
Total bacteria	17	53	393	463	84.9	–	0.51	0.011*

*Number of unique genes, shared genes, and total gene number, shared abundance (%), SIMPER dissimilarity index (%), and BRO11/BRO12 ratio are indicated. Statistically significant differences in terms of total number of genes between BRO11 and BRO12 are indicated with *$p < 0.05$, **$p < 0.01$.*

15 in acetogenesis, 856 in denitrification, 284 in nitrification, 255 in nitrogen fixation, 134 in assimilatory N reduction, 130 in dissimilatory N reduction, 271 in ammonification, 3 anammox, 973 in sulfur cycling, 342 in phosphorus cycling, 5,892 in organic compound remediation, 5,036 in stress process, 258 in energy process, 5,649 in metal resistance, 889 in antibiotic resistance, 858 in fungi-related activities, 1,165 in pathogens and virus, and 261 in other categories. The results revealing the most relevant differences are indicated in **Figure 4**.

For each category, detected genes were analyzed by hierarchical clustering (not shown). Among the gene categories involved in biogeochemical cycles, genes related to carbon and nitrogen cycles were the most abundant. Considering that BRO11 samples showed about 12% more genes than BRO12, it is not surprising that most of the gene categories display significant higher values in BRO11 than in BRO12. Thus, besides C and N gene categories, we specifically focused the analyses on functional genes categories that highlighted contrasting patterns among BRO11 and BRO12 compared the overall distribution (i.e., sulfide oxidation category).

Carbon cycling is one of the most important and complex microbial-driven process occurring in soils. Here, a total of 2,788 genes involved in carbon cycling were detected, of which 2,164 were related to carbon degradation and 1,800 were shared between BRO11 and BRO12 soils. Overall, BRO11 showed the highest number of detected unique genes (267) compared to BRO12 (97). Among all the detected genes, carbon degradation is the most important gene category and includes several sub-categories specific for distinct organic substrates. As expected, BRO11 showed a greater number of total genes involved in C degradation than BRO12 (+8.9%, $p < 0.05$). In particular, the higher differences occurred in cellulose (+11.5%, $p < 0.01$), lignin (+12.6%, $p < 0.01$), chitin (+12.8%, $p < 0.05$), and starch (+8.6%, $p < 0.05$) sub-categories (**Table 3**). However, most of the genes of such sub-categories were shared among the two vineyards: starch (456/543), cellulose (159/190), hemicellulose (285/337), chitin (262/336), lignin (176/211), and pectin (12/16) (**Table 3**). Furthermore, the key enzymes involved in C fixation (CODH, pcc, rubisco, adB) are shared among the two vineyards and did not show any statistical difference.

Considering the nitrogen cycle, as already observed for C degradation, BRO11 soils displayed a greater number of total genes involved in N cycle than BRO12 (+15.8%, $p < 0.05$). A total of 1912 gene probes involved in nitrogen fixation (255), denitrification (856), nitrification (277), dissimilatory N reduction (130), assimilatory N reduction (134), ammonification (257), and anaerobic ammonium oxidation (3) were detected in BRO11 and BRO12 soils and most of them (79.5%) resulted overlapping (**Table 3**). Interestingly, the least shared gene number among BRO11 and BRO12 was detected in assimilatory (nirA and nirB) N reduction gene sub-category, which showed the higher statistical difference (+33.3%, $p < 0.01$ and +28.6%, $p = 0.001$, respectively; **Table 3**). Significant differences between BRO11 and BRO12 occurred also in nitrogen fixation and denitrification sub-categories, which are considered among the most important processes in nitrogen cycling. In the first case, the majority of *nifH* genes (83.5%) were shared among BRO11 and BRO12, and sub-category exhibited the highest BRO11/BRO12 ratio value (0.76), indicating that most of the unique *nifH* genes were found in BRO11 soils (+14.7%, $p < 0.01$). Similarly, some of the genes involved in denitrification such as *nirK* and *nirS* were more abundant in BRO11 soils (+9.8%, $p < 0.05$ and +13.1%, $p < 0.05$, respectively; **Table 3**). Moreover, also the total number of *nifH* and *nirA* genes resulted significantly higher in BRO11 than in BRO12 (+24.2%, $p = 0.002$ and +36.3%, $p = 0.001$, respectively). On the other hand, most of the functional genes involved in denitrification were shared among BRO11 and BRO12 (718/856). Among them, nirK and nirS genes provided the highest values of BRO11/BRO12 ratio (0.59 and 0.67, respectively), while narG, norB, and nosZ had

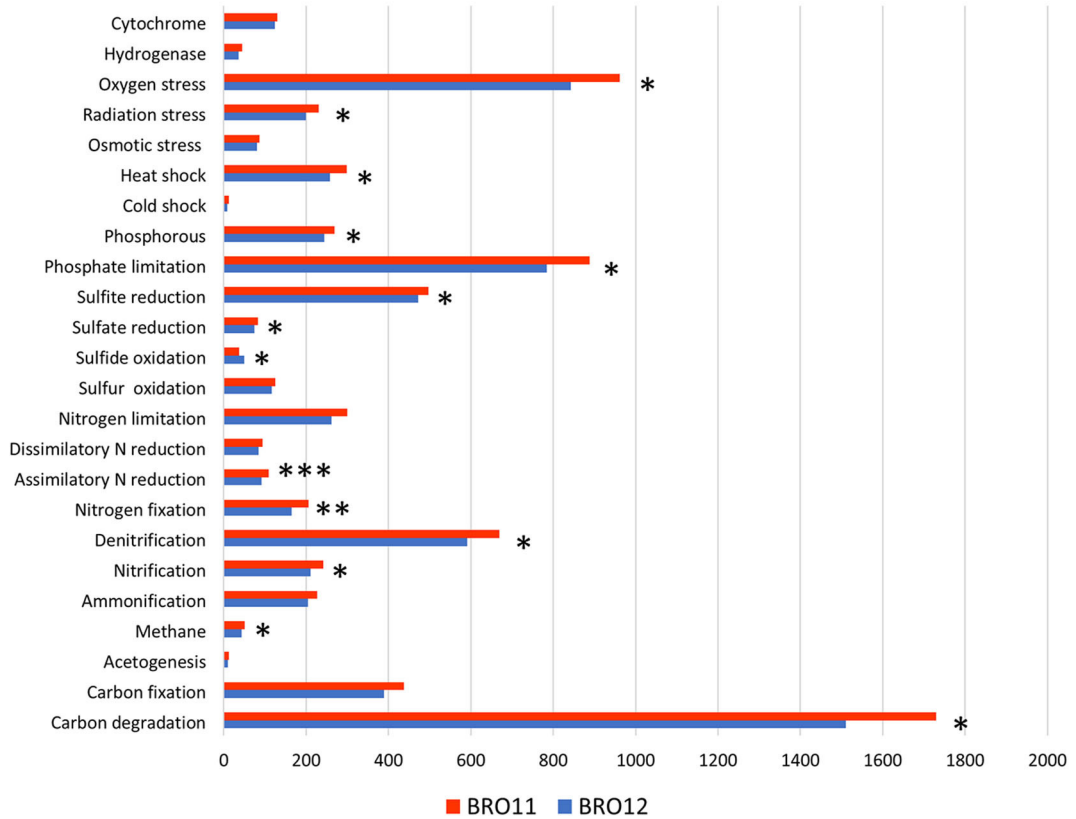


FIGURE 4 | Results of metagenomic analysis of soils BRO11 and BRO12 by means of Geochip 3.0. The total number of the detected functional genes is reported for each gene category. Asterisks indicate significant differences of the variable means between BRO11 and BRO12 genes (*$p < 0.05$; **$p < 0.01$; and ***$p < 0.001$).

lower values (0.40, 0.33, and 0.33, respectively). Interestingly, *nirK* and *nirS* were more represented in BRO11 than in BRO12 site (+24.0% and +16.5%, respectively; $p < 0.05$)Despite the low values of both Simper dissimilarity index and BRO11/BRO12 ratio, genes involved in ammonification (amoA) are significantly more abundant in BRO11 (+14.5%; $p < 0.05$).

No significant differences occurred in the other nitrogen sub-categories.

Among all the other gene categories included in Geochip 3.0, the most interesting results have been found in the sulfur cycling (**Table 3**). Most of the detected genes (826/973) in this category were shared among BRO11 and BRO12, and mainly related to sulfate and sulfite reduction processes (*aprA*, *APS_AprAB*, *dsrA, dsrB, cysJ, sir*). Hierarchical clustering analysis highlighted two groups of genes with contrasting values among BRO11 and BRO12. As shown in **Figure 5**, four different patterns were observed (indicated with letters A, B, C, and D): green and red color indicates all the detected genes with signal intensity below and above the average value calculated for this category, respectively. Thus, pattern A includes genes with high signal intensity in BRO11 and BRO12 soils, whereas pattern D includes genes with low signal intensity in both of them. Most of the detected genes clustered in group B were dsrA and dsrB and exhibited higher intensity in BRO11 compared to BRO12. On the other hand, in group C the highest signal intensity was provided by genes cysJ and, in minor extent, fccAB, dsrA, dsr B, and sox in BRO12 (**Figure 5**). Remarkably, an overrepresentation of genes involved in sulfate reduction was observed in BRO11, including *aprA* gene which encodes the subunit A of the adenylylsulfate reductase (EC 1.8.99.2, K00394), and *dsrA* and *dsrB* genes encoding subunits A and B of the sulfate reductase (EC 1.8.99.3, K11180, and K11181). As also indicated by the highest Simper value and BRO11/BRO12 ratio, dsrA gene was significantly more represented in BRO11 than in BRO12 (+21.4%, $p < 0.001$). Nevertheless, BRO12 exhibited an unusual high number of fccAB (+29.9%, $p < 0.01$) and sqr (+40%, $p < 0.05$) genes involved in sulfide oxidation compared to BRO11.

Enzymatic Analyses

In general, BRO11 soils showed higher enzymatic activities than BRO12 (**Figure 6**). The activity of alkaline phosphatase (alkP) showed the highest values (180 pmol/g soil), whereas the lowest were found for arylsulfatase (8 pmol/g soil). With this regard, the higher arylsulfatase activity ratio revealed that, among the considered enzymes the

TABLE 3 | The detected gene probes involved in carbon, nitrogen, and sulfur cycling and their main sub-categories.

Gene category (genes)	Unique BRO12	Unique BRO11	Shared	Tot	Shared %	Simper %	BRO11/ BRO12 ratio	*P*-value
C degradation	97	267	1,800	2,164	83.2	–	0.47	0.019*
Cellulose	6	25	159	190	83.7	11.9	0.61	0.002**
Chitin	19	55	262	336	78	20.6	0.49	0.037*
Hemicellulose	20	32	285	337	84.6	10.4	0.23	0.081
Lignin	6	29	176	211	83.4	13.6	0.66	0.007**
Starch	23	64	456	543	84	23.5	0.47	0.018*
Pectin	2	2	12	16	75	1.1	0	0.069
Others	21	60	450	531	84.7	19.1	0.48	0.038*
Nitrogen cycle	70	322	1,520	1,912	79.5	–	0.64	0.030*
N fixation (NifH)	5	37	213	255	83.5	20.3	0.76	0.002**
Nitrification (amoA)	9	25	243	277	87.7	14.9	0.47	0.021*
Dissimilatory N reduction (napA, nrfA)	9	19	102	130	78.5	3.1	0.36	0.19
Denitrification (narG)	22	51	377	450	83.8	16.7	0.40	0.091
Denitrification (nirK)	6	23	124	153	81	11.7	0.59	0.016*
Denitrification (nirS)	3	15	119	137	86.9	7.6	0.67	0.038*
Denitrification (norB)	1	2	33	36	91.7	1.3	0.33	0.579
Denitrification (nosZ)	5	10	65	80	81.3	3.5	0.33	0.139
Denitrification tot	37	101	718	856	83.9	40.8	0.46	0.054
Assimilatory N reduction (nasA)	2	3	45	50	90	1.6	0.20	0.374
Assimilatory N reduction (niR)	1	2	46	49	93.9	2.3	0.33	0.025*
Assimilatory N reduction (nirA)	1	5	11	17	64.7	2.2	0.67	0.008**
Assimilatory N reduction (nirB)	0	4	14	18	77.8	2.8	1.00	0.001***
Assimilatory N reduction tot	4	14	116	134	86.6	8.9	0.56	0.011*
Anammox (hzo)	0	0	3	3	100	0.2	0	0.374
Ammonification (ureC)	4	22	224	250	89.6	10.2	0.69	0.089
Ammonification (gdh)	2	2	3	7	42.9	1.4	0	0.349
Ammonification tot	6	24	227	257	88.3	11.6	0.60	0.104
Sulfur cycle	61	86	826	973	84.9	–	0.17	0.381
Adenylylsulfate reductase (aprA)	0	4	30	34	88.2	2.4	1.00	0.115
Adenylylsulfate reductase (APS-AprA)	1	3	40	44	90.9	2.7	0.50	0.448
Adenylylsulfate reductase (APS-AprB)	1	5	19	25	76	3.5	0.67	0.041*
Sulfite reductase (CysJ)	12	3	146	161	90.7	15.6	0.60	0.045*
Sulfite reductase (dsrA)	6	38	206	250	82.4	35.2	0.73	0.001***
Sulfite reductase (dsrB)	12	20	154	186	82.8	10.3	0.25	0.108
Sulfite reductase (sir)	10	0	50	60	83.3	10.5	−1.00	0.009**
Sulfide oxidation (fccAB)	9	0	41	50	82	8.1	−1.00	0.004**
Sulfide oxidation (sqr)	2	0	5	7	71.4	2.1	−1.00	0.039*
Sulfur oxidation (sox)	8	13	135	156	86.5	9.7	0.24	0.339

*Number of unique genes, shared genes and total gene number, shared abundance (%), SIMPER dissimilarity index (%), and BRO11/BRO12 ratio are indicated. Statistically significant differences in terms of total number of detected genes are indicated with *$p < 0.05$, **$p < 0.01$, or ***$p < 0.001$.*

arylsulfatase is the most present in BRO11. This result is consistent with the higher sulfate content in the Ap horizon of the BRO11 soil (7.26 ppm) compared to BRO12 (6.22 ppm).

The total amount of dsDNA extracted from the two soil with the same procedure used for enzymes was significantly different. Specifically, the extraction yield of the DNA varied among 1.8–15 μg/g soil in BRO11, while it was <0.2 in all the BRO12 samples. This data is consistent with the higher gene number detected in BRO11.

DISCUSSION

In the present study, we examined the microbial communities of similar soils giving rise to wines of highly disparate qualities to assess the relevance of the "microbial terroir" concept.

Thus, to figure out the role of soil microbiota and its functions in determining grape and wine quality, it was essential to minimize the environmental variability by conducting research at a within-vineyard scale, to compare microbial features among really similar soils. It does not just mean to select

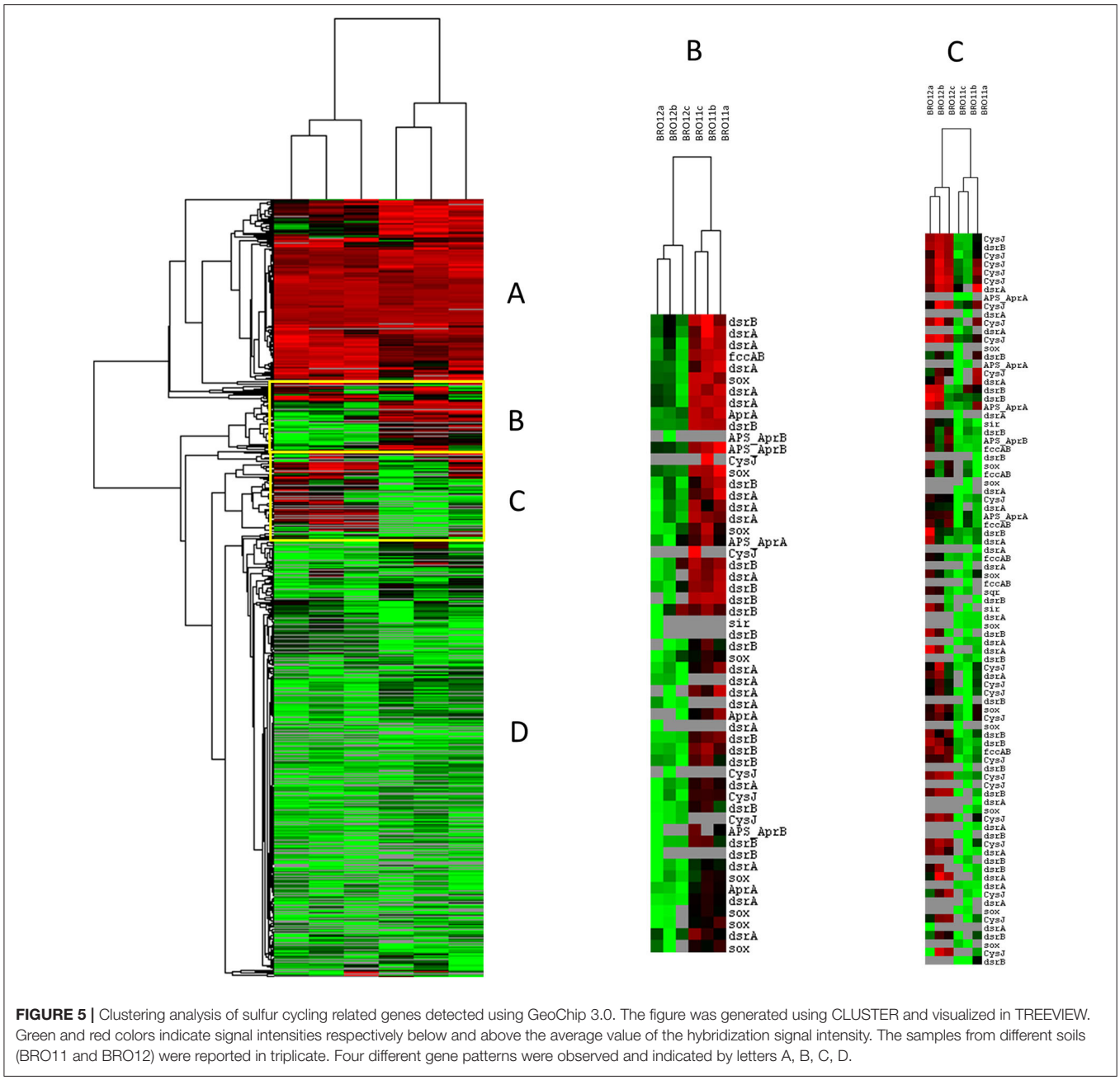
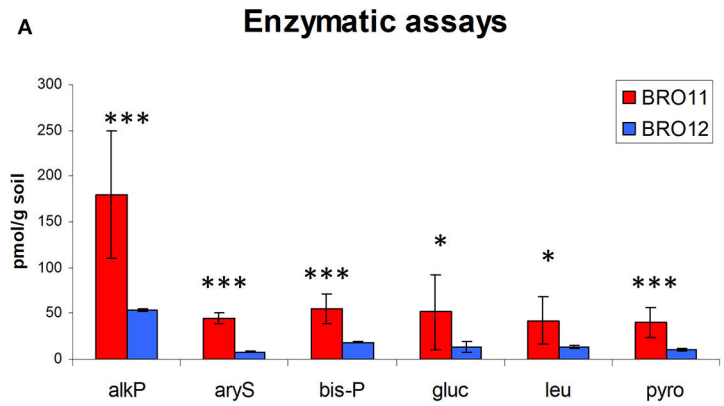


FIGURE 5 | Clustering analysis of sulfur cycling related genes detected using GeoChip 3.0. The figure was generated using CLUSTER and visualized in TREEVIEW. Green and red colors indicate signal intensities respectively below and above the average value of the hybridization signal intensity. The samples from different soils (BRO11 and BRO12) were reported in triplicate. Four different gene patterns were observed and indicated by letters A, B, C, D.

soils belonging to the same "soil type" category, according with the IUSS taxonomy, but it is necessary a preliminary pedological survey to assess the spatial soil heterogeneity to support soil sampling. In fact, the high spatial and temporal heterogeneity of most of soil properties and processes may vary from nano- to macroscales. For example, the physical and chemical properties might change even within soils of the same IUSS class, and the of input of labile organic compounds released by roots creates microbial hotspots over short periods of time (Kuzyakov and Blagodatskaya, 2015). Such differences in terms of soil structure, pH, nutrients availability, etc. may strongly affect the soil microbiota, both in terms of diversity and function (Fierer and Jackson, 2006; Kuramae et al., 2012; Yan et al., 2019). Nevertheless, to date most of the studies conducted to assess any correlation between soil microbiota and grapevine health and wine quality have been carried out on different sites or soils, regardless of soil heterogeneity. For example, a previous study about the composition of the soil microbiota in 19 vineyards selected from sub-appellations of the GI Napa Valley AVA, an American Viticultural Area located in California, USA has found different soil microbial community structures (Burns et al., 2015). However, the authors could not state whether the differences were merely correlated with the AVA features or if the microbiota had a direct effect on vine



B **Activity ratio (BRO11/BRO12)**

Phosphomonoesterase (alkP)	3,3
Arylsulfatase (aryS)	5,5
Phosphodiesterase (bis-P)	3,0
β-glucosidase (gluc)	3,8
Leucine-aminopeptidase (leu)	3,1
Pyroposphatase (pyro)	4,1
dsDNA	36,0

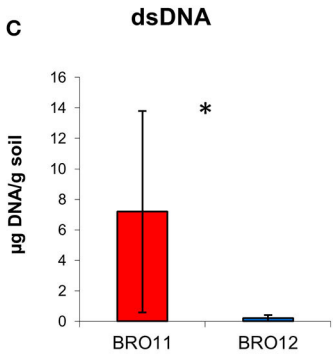


FIGURE 6 | (A) Enzymatic activities detected in BRO11 and BRO12 soils and the amount of dsDNA extracted with the same procedure used for enzymes. Asterisk indicates significant differences of the variable means between BRO11 and BRO12 genes ($p < 0.05$); **(B)** the activity ratio among BRO11/BRO12 values indicate the relative enzymatic activity and dsDNA amount in the two soils. **(C)** Total amount of the dsDNA extracted from BRO11 and BRO12 soils. Asterisks indicate significant differences of the variable means between BRO11 and BRO12 sites (*$p < 0.05$; and ***$p < 0.001$).

health and/or grape quality. Bokulich et al. (2016) showed that the wine grape-associated microbiota correlate with vineyard and pedoclimatic conditions and suggested that the grape microbiome was reflected in the fermentations thus influencing the wine qualitative traits. Similarly, another study reported that most of the bacterial communities associated with vine plants exhibited patterns highly reflective of the surrounding native soil microbiota, thus highlighting the importance of both local soil pedoclimatic factors and vineyard management in shaping their composition and structure (Zarraonaindia et al., 2015). Recently, a similar conclusion was reported by Vadakattu et al. (2019), who showed how distinct soil bacterial and fungal communities were correlated to the aroma of Shiraz grapes within the same vineyard in the Grampians region of Victoria (Australia); however, they could not detect any specific functional trait of the microbial members that accounted for these differences.

As a first study of this type, here we investigated the functional diversity of soil microbiota occurring at within-vineyard scale, after surveying soils with proximal sensors on BRO11 and BRO12 sites and confirming that such soils were very similar and spatially homogeneous, especially in the Ap horizon (0–40 cm), where soil samples for microbiological analyses were collected. Although this approach allowed us to minimize the soil variability and to link the microbial community structure and functions with grape and wine quality, the fact that we compared only two plots within the same vineyard (although with replications within the sections of the field) might represent also a limitation. In fact, more analyses should be carried out on a higher number of vineyards to make these data more robust. Another limitation might be related to the small differences in soil parameters of BRO11 and BRO12, which are similar but not identical (see **Table 1** and **Table S1**), that might also directly affect grape quality. For example, the sand content of BRO11 is lower than in BRO12. Similarly, $CaCO_3$, total nitrogen, total sulfate, and SOC availability in BRO12 soil were much lower than in BRO11. Moreover, in our previous research conducted on such soils to monitor the water stress over 3 years (2008-′09-′10), we found out that the grapevine water stress, determined by means of the ratio occurring among the two stable carbon isotopes $^{13}C/^{12}C$ ($\delta^{13}C$), was moderately higher in BRO11 despite the soil moisture monitoring provided similar results in both the profiles (Costantini et al., 2013). A possible explanation to the lower grapevine water stress of BRO12 is the shallower limit between sandy deposits, very permeable, and the underlying clayey deposits, characterized by slow water permeability. This boundary is suitable for the formation of a temporary water table or for higher soil moisture, also during dry

season. According to the electrical resistivity tomography (ERT), reported by the study of Martini et al. (2013), this limit is about 10 m deep in the area of BRO11 and about 4–5 m in BRO12. Although it is unlikely that vine rooting system in BRO12 reach the water table at 4 meters depth, it is possible that the subsoil remains more humid than in BRO11. In any case, such scenario should not significantly affect the microbial community structure and activity in the topsoil, at 0–30 cm depth.

The GeoChip 3.0 microarray analyses revealed that the average gene number detected in BRO11 was about 12% higher than in BRO12. This might be likely due to the higher SOC content and the higher native dsDNA content in BRO11 soil. However, although most of the detected genes were shared among the two soils, in general they presented a different gene pattern distribution, highlighting distinct microbial traits among BRO11 and BRO12 soils. Thus, we have focused our attention mainly on the statistically significant differences occurring among the two soils and the unique phylogenetic and functional features associated to BRO11 or BRO12.

The phylogenetic structure of the microbial community based upon gyrB revealed an enrichment of bacterial taxa belonging to Actinobacteria in BRO12, whereas an increase of Alfa- and Gamma-Proteobacteria occurred in BRO11 soils. Among Actinobacteria, some of the detected taxa such as *Rubrobacter xylanophilus* DSM 9941 and *Mycobacterium smegmatis* str, MC2 155 are known to accumulate and synthetize trehalose, an organic compound able to protect bacteria from several environmental stress such as heat, salinity, oxidation, radiation, and desiccation (Nobre et al., 2008). Interestingly, threalose was reported to play as both a thermoprotectant and a precursor of critical cell wall metabolites. Thus, as many bacteria were shown to use trehalose as major organic osmoprotectant and/or thermoprotectant (Woodruff et al., 2004), we speculate that the enrichment of bacteria such as *Rubrobacter xylanophilus* DSM 9941 and *Mycobacterium smegmatis* str, MC2 155 might be related to the higher water and desiccation stress conditions found in BRO12 compared to BRO11.

On the other hand, among the bacterial taxa belonging to Alpha-Proteobacteria, most of the unique organisms detected in BRO11 belonged to Rhodobacter (i.e., *R. sphaeroides* and *R. capsulatus*) and Rhodospirillum (i.e., *R. centenum* and *R. rubrum*) genera or to Rhizobiales order (i.e., Bradyrhizobium, Mesorhizobium, and Methylobacterium genera) which are known to be involved in nitrogen-fixation process (Masepohl et al., 2002; Zhang et al., 2005; Carvalho et al., 2010), thus promoting the nitrogen availability in BRO11 compared to BRO12 soil.

Interestingly, most of the microbial taxa exclusively detected in BRO11 soil and belonging to Gamma-Proteobacteria have been reported to display extracellular electron transfer metabolism they can use under strictly anaerobic or microaerophilic conditions (i.e., *Shewanella* sp., *Alcanivorax* sp., *Enterobacter* sp., etc.) as well as *Geobacter sulfurreducens* among Delta-Proteobacteria. Such peculiar features have been reported to be potentially interesting for biotechnological purposes (Logan, 2009), but also as key mechanisms for the humification of soil organic matter (Mocali et al., 2013). Hence, their enrichment in BRO11 soil might indicate more reducing conditions than in BRO12. The higher presence of *Acidithiobacillus ferrooxidans* in BRO12 is likely related to its ability to oxidize iron and various reduced inorganic sulfur compounds as energy sources, thus promoting the bioleaching and the extraction of such metals from soil. More specifically, despite its ability to use sulfur as substrate to form thiosulfate $S_2O_3^{2-}$ thus promoting sulfur oxidation, the Sox multienzyme-complex is absent in *A. ferrooxidans* (Janosch et al., 2015). It worth mentioning the enrichment in *Sulfobacillus thermosulfidooxidans* sp. in BRO11, a thermophilic and facultative anaerobe bacteria related to Firmicutes and capable of reversible oxidation of sulfur-containing compounds. Interestingly, *S. thermosulfidooxidans* displays similar functional properties than *A. ferrooxidans*, which strongly suggests that they may share the similar niche. However, the model of sulfur oxidation in *S. thermosulfidooxidans* has some different characteristics from the sulfur oxidation of *A. ferrooxidans*. In fact, it was reported that the genes involved in direct or indirect sulfite oxidation pathway are missing in *S. thermosulfidooxidans* whereas in *A. ferrooxidans* sulfites may be converted to adenosine-5′-phosphosulphate (APS) and then oxidized to sulfate via an indirect pathway controlled by APS reductase (Guo et al., 2014).

Regarding the carbon cycle, the main significant differences between BRO11 and BRO12 occurred among the genes involved in C degradation. In general, more than 80% of the genes were overlapped among the two soils and most of the unique genes occurred in BRO11, thus indicating a greater degradation potential occurring in such soil, likely due to the higher content of organic matter and microbial biomass. More specifically, BRO11 displayed higher number of genes related to the subcategories degrading chitin and starch, which showed the highest contribution to explain the differences between BRO11 and BRO12 (**Table 3**), according to the Simper values. However, the highest statistical differences occurred in cellulose and lignin subcategories, also in according to the BRO11/BRO12 ratio. In general, this suggests a greater mineralisation rate of organic compounds in BRO11 soils, thus providing a higher availability of nutrients than in BRO12.

Concerning the nitrogen cycling, 79.5% of the total genes (1,520/1,912) were shared among BRO11 and BRO12, whereas most of the unique genes were detected in BRO11 soil samples. The main differences occurred in the nitrogen fixation and denitrification sub-categories, also according to the BRO11/BRO12 ratio index (**Table 3**); moreover, according to the Simper values, together they contributed for 71.3% of the total dissimilarity among BRO11 and BRO12 (**Table 3**). This result is consistent with the hypothesis that BRO11 soils provide more reducing conditions than BRO12. Interestingly, as oxygen-limiting condition in soil is known to promote both N mineralization (Ono, 1989) and the activity of nitrogen-fixing bacteria, it might also be related to the greater N availability in BRO11 than in BRO12. It is important to note that in the production of red table wines, moderate nitrogen availability is one of the main factors in determining the plant health and, ultimately, the wine quality. In fact, low nitrogen supply limits berry size and vine vigor, and it increases sugar content,

anthocyanins, and phenolic content, whereas excessive nitrogen supply is not desired because it increases susceptibility of grapes to gray rot (Soubeyrand et al., 2014; van Leeuwen et al., 2018). Because nitrogen also stimulates the synthesis of glutathione (a compound that preserves aroma compounds in musts and wines) and limits the production of tannins (that are involved in volatile thiol degradation), moderately high nitrogen supply to the vines is desired in wine production only in varieties dependent on volatile thiols for their aromatic signature (Choné et al., 2006).

Regarding the differences among the other functional gene categories, in general the two soils presented a very similar gene pattern distribution. Overall, BRO11 showed a higher number of detected genes than BRO12 except for genes involved in S cycling category, where a significant enrichment of sulfur- and sulfide-oxidation genes was observed in BRO12. These results suggest that S cycling, might play a key role in determining plant health and, ultimately, wine quality. Moreover, such results are consistent with the higher content of total sulfate found in BRO11 and the higher presence of sulfate transporters provided by fungi such as *Sclerotinia sclerotiorum* 1980 and *Aspergillus terreus* NIH2624 detected only in BRO11 soil, indicating a higher sulfate availability in BRO11 soil compared to BRO12. The enrichment of genes involved in sulfur oxidation in BRO12 may be due to the activities of chemolithotrophic sulfur-oxidizing bacteria such as *Paracoccus denitrificans, Thiobacillus denitrificans*, or *Acidithiobacillus ferrooxidans,* which are metabolically very flexible and capable to uptake CO_2 and inorganic sulfur to support both cell basal metabolism and the acquisition of energy (Tang et al., 2009; Pokorna and Zabranska, 2015), but also to use denitrification process under oxygen-limited conditions (*P. denitrificans* and *T. denitrificans*) or to perform the solubilisation of minerals by bioleaching activity (*A. ferrooxidans*). *P. denitrificans* and *T. denitrificans* were more abundant in BRO11, where nir genes (niR, nirA, nirB, nirK, and nirS) were significantly enriched, confirming a greater denitrification process compared to BRO12. On the other hand, *A. ferrooxidans* resulted more enriched in BRO12, thus promoting the oxidation of sulfur to thiosulfate and, ultimately, to sulfate (Guo et al., 2014; Janosch et al., 2015). This result is consistent with the overexpression of sqr and fccAB genes detected in BRO12, which are involved in the sulfur and sulfide oxidation pathways, thus indicating an enhancement of oxidative reactions in S cycling of BRO12 soils.

The high BRO11/BRO12 ratio value of the arylsuphatase activity confirm a greater sulfate turnover in BRO11 than in BRO12 soils. Arylsulphatase is known to be an important enzyme promoting the hydrolysis of sulfate esters in the soil, and the ability to mobilize sulfate esters is extremely important for the

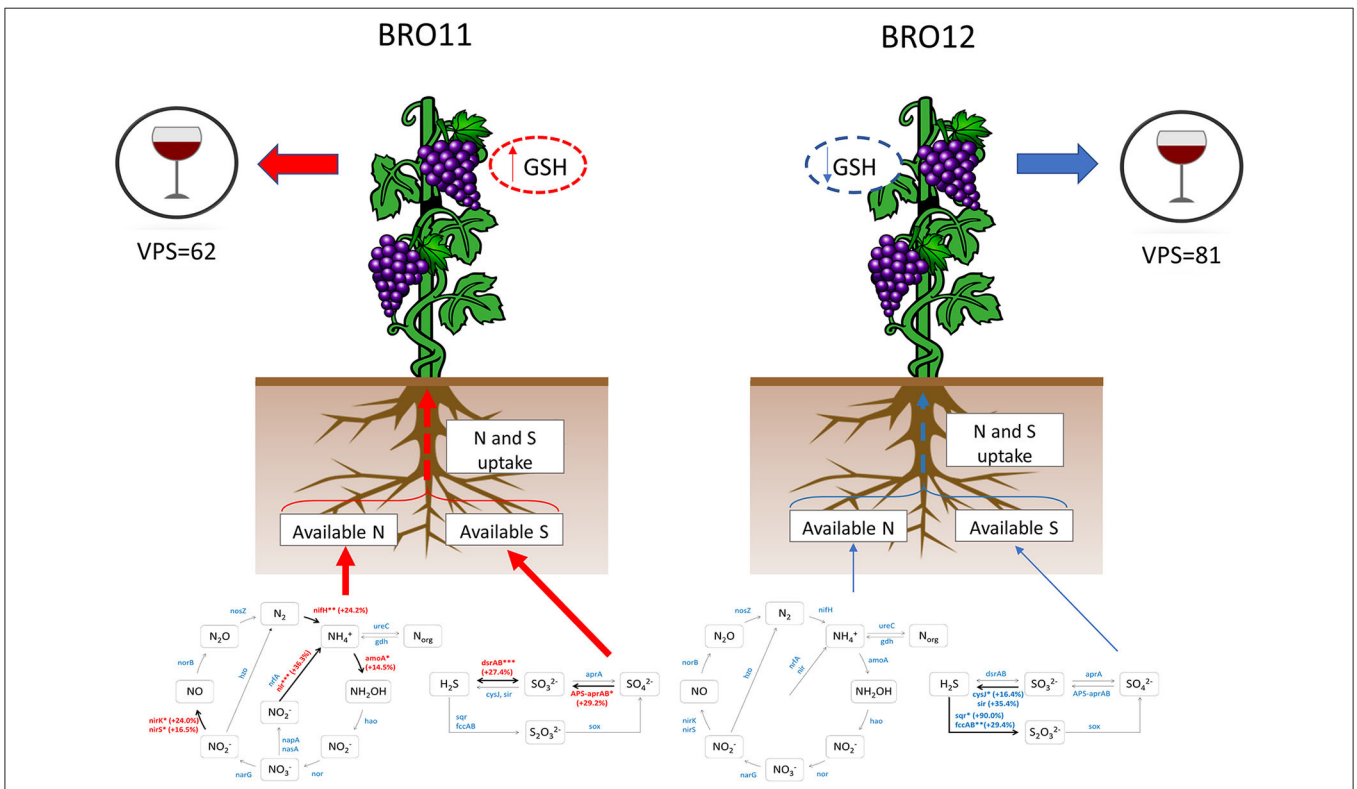


FIGURE 7 | Schematic overview of the potential effects of the different nitrogen and sulfur availability on the plant physiology and wine quality of BRO11 and BRO12 plots. On BRO11 the higher availability of sulfate and nitrogen is supposed to enhance the plant uptake of such nutrients, thus promoting the synthesis of reduced glutathione (GSH) and reducing the phenolic compounds which determine the final wine quality (VPS). Dashed lines indicate the hypothetic effect whereas the reduced arrow's thickness in BRO12 indicates a minor amount of available nitrogen ad sulfate.

survival of many soil bacterial species but it is also critical to promote plant growth (Kahnert et al., 2002; Kertesz and Mirleau, 2004). Since plants are able to uptake sulfur primarily as inorganic sulfate, reducing it to S^{2-} and incorporating it into cysteine for protein synthesis, the most important form of sulfur for plant nutrition is SO_4^{2-} (Wilhelm Scherer, 2009). Consequently, the efficient use of carbon and nitrogen in plant growth depends also on the absorption and assimilation of appropriate amounts of sulfate (Kertesz et al., 2007). Therefore, sulfur deficiency in BRO12 soils may have seriously inhibited nitrogen fixation, in accordance with literature (i.e., Anderson and Spencer, 1950), thus reducing the available nitrogen for plants.

Interestingly, plants can uptake SO_4^{2-} molecules not only for protein synthesis. For example, the reduced glutathione (GSH) is a linear tripeptide constituted by glycine, glutamate and cysteine which exerts several activities in must and wine (Kritzinger et al., 2012). In fact, high GSH content in grapes is a key factor in aroma protection in several wine varieties and plays an important role in protecting varietal volatile thiols from oxidation in musts and wines (Lacroux et al., 2008). The first source of GSH is the grape, where it can exceed 200 mg/L of grape juice according to plant cultivar, pedoclimatic conditions and agronomic practices and the amount of available nitrogen in the soil (Fracassetti and Vigentini, 2018). It is well-known that a higher availability of nitrogen leads to an increase of GSH levels in berries and grape juice but also to a decrease of phenolic compounds, which are important factors for determining some wine traits and preserving volatile thiols during grape processing (Choné et al., 2006). Therefore, in addition to the well-established effects due to nitrogen limitation, we suggest that the reduced availability of nitrogen and sulfate in BRO12 soil might have reduced the GSH levels in berries and increased the amount of phenolic compared to the grapes deriving from BRO11 site. This hypothesis is also consistent with the overall VPS values related to wine quality (BRO11 = 62, BRO12 = 81) determined in our previous work (Costantini et al., 2013), as one of the main parameters used to calculate the VPS values is the phenolic compounds content (Bucelli et al., 2010; **Figure 7**).

CONCLUSION

In this work, we assessed a possible effect of soil microbiota on wine quality through a functional approach at vineyard-scale. The metagenomic approach by Geochip 3.0 not only confirmed the key role of nitrogen availability in shaping grape and wine quality but also highlighted the potential role of the sulfur metabolism as another determining co-factor in vineyard-scale variation among wine grapes. Overall, the results represent a starting point to better unravel the complexity of plant-soil-microbial interactions behind the terroir concept supporting the existence of a "*microbial functional terroir*" effect. In fact, functional—rather than just genetic—microbial diversity might play a key role in the development of novel approaches in viticulture. For example, these results might open the door to new approaches in viticulture, aiming to "modulate" the plant phenotype and its related products throughout specific manipulation of plant-associated soil microbiota metabolism rather than their composition. Some recent results showed the importance of plant-associated microorganisms in regulating the plant phenotype, proposing "to approach microbiota as modulators of plant phenotype" and providing effects with strong similarities with breeding (Ravanbakhsh et al., 2019). Therefore, our results have potentially important implications for (precision) viticulture, grape growers and winemakers to better understand the potential role of the native soil microbiota in vineyards to satisfy the growing demand for high grape yield and improving specific wine traits, thus exalting the typicality and the characteristics of every single plot within the vineyard.

DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found in the Gene Expression Omnibus (GEO) of the NCBI (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE146289).

AUTHOR CONTRIBUTIONS

SM and SP defined the experimental design and collected the soil samples. SM prepared and conducted the GeoChip analysis and did the statistical analysis. SP carried out the pedological surveys and the soil characterization. EK and GK contributed to the discussion of molecular data. FF did the enzymatic assays. All the authors contributed to prepare the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2020.00075/full#supplementary-material

REFERENCES

Anderson, A. J., and Spencer, D. (1950). Sulphur in nitrogen metabolism of legumes and non-legumes. *Aust. J. Biol. Sci.* 3, 431–449. doi: 10.1071/BI9500431

Andrenelli, M. C., Fiori,V., and Pellegrini, S. (2013). Soil particle-size analysis up to 250 μm by X-ray granulometer: device set-up and regressions for data conversion into pipette-equivalent values. *Geoderma* 192, 380–393. doi: 10.1016/j.geoderma.2012.06.011

ASTM International (2015). *C1580-09e1: Stand and Test Method for Water-Soluble Sulfate in Soil*. West Conshohocken, PA: American Society for Testing and Materials.

Barata, A., Malfeito-Ferreira, M., and Loureiro, V. (2012). The microbial ecology of wine grape berries. *Int. J. Food Microbiol.* 153, 243–259. doi: 10.1016/j.ijfoodmicro.2011.11.025

Belda, I., Zarraonaindia, I., Perisin, M., Palacios, A., and Acedo, A. (2017). From vineyard soil to wine fermentation: microbiome approximations to explain the "terroir" concept. *Front. Microbiol.* 8:821. doi: 10.3389/fmicb.2017.00821

Bokulich, N. A., Collins, T. S., Masarweh, C., Allen, G., Heymann, H., Ebeler, S. E., et al. (2016). Associations among wine grape microbiome, metabolome, and fermentation behavior suggest microbial contribution to regional wine characteristics. *MBio* 7:e00631-16. doi: 10.1128/mBio.00631-16

Bokulich, N. A., Thorngate, J. H., Richardson, P. M., and Mills, D. A. (2014). Microbial biogeography of wine grapes is conditioned by cultivar, vintage, and climate. *Proc. Natl. Acad. Sci. U.S.A.* 111, E139–E148. doi: 10.1073/pnas.1317377110

Braschi, E., Marchionni, S., Priori, S., Casalini, M., Tommasini, S., Natarelli, L., et al. (2018). Tracing the 87Sr/86Sr from rocks and soils to vine and wine: an experimental study on geologic and pedologic characterisation of vineyards using radiogenic isotope of heavy elements. *Sci. Total Environ.* 628, 1317–1327. doi: 10.1016/j.scitotenv.2018.02.069

Bucelli, P., Costantini, E. A. C., and Storchi, P. (2010). It is possible to predict Sangiovese wine quality through a limited number of variables measured on the vines. *OENO One* 44, 207–218. doi: 10.20870/oeno-one.2010.44.4.1473

Burns, K. N., Kluepfel, D. A., Strauss, S. L., Bokulich, N. A., Cantu,D., and Steenwerth, K. L. (2015). Vineyard soil bacterial diversity and composition revealed by 16S rRNA genes: differentiation by geographic features. *Soil Biol. Biochem.* 91, 232-−247doi: 10.1016/j.soilbio.2015.09.002

Capozzi, V., Garofalo, C., Chiriatti, M. A., Grieco, F., and Spano, G. (2015). Microbial terroir and food innovation: the case of yeast biodiversity in wine. *Microbiol. Res.* 181, 75–83. doi: 10.1016/j.micres.2015.10.005

Carvalho, F. M., Souza, R. C., Barcellos, F. G., Hungria, M., and Vasconcelos, A. T. R. (2010). Genomic and evolutionary comparisons of diazotrophic and pathogenic bacteria of the order Rhizobiales. *BMC Microbiol.* 10:37. doi: 10.1186/1471-2180-10-37

Choné, X., Lavigne-Cruège, V., Tominaga, T., van Leeuwen, C., Castagnède, C., Saucier, C., et al. (2006). Effect of vine nitrogen status on grape aromatic potential: flavor precursors (S-cysteine conjugates), glutathione and phenolic content in *Vitis vinifera* L. Cv Sauvignon blanc grape juice. *OENO One* 40, 1–6. doi: 10.20870/oeno-one.2006.40.1.880

Costantini, E. A. C., Agnelli, A., Bucelli, P., Ciambotti, A., Dell'Oro, V., Natarelli, L., et al. (2013). Unexpected relationships between δ 13 C and wine grape performance in organic farming. *OENO One* 47, 269–285.doi: 10.20870/oeno-one.2013.47.4.1556

Cowie, A. L., Lonergan, V. E., Fazle Rabbi, S. M., Fornasier, F., Macdonald, C., Harden, S., et al. (2013). Impact of carbon farming practices on soil carbon in northern New South Wales. *Soil Res.* 51, 707–718. doi: 10.1071/SR13043

Deloire, A., Vaudour, E., Carey, V. A., Bonnardot, V., and Van Leeuwen, C. (2005). Grapevine response to terroir: a global approach. *J. Int. Sci. Vigne Vin.* 39–34, 149–162. doi: 10.20870/oeno-one.2005.39.4.888

Eisen,M., Spellman,P., Brown,P., and Botstein,D. (1998). Cluster analysis and display of genome-wide expression patterns. *Proc. Natl Acad. Sci. U.S.A.* 95, 14863–14868. doi: 10.1073/pnas.95.25.14863

Felder, D., Burns, D., and Chang, D. (2012). Defining microbial terroir: the use of native fungi for the study of traditional fermentative processes. *Int. J. Gastr. Food Sci.* 1, 64–69. doi: 10.1016/j.ijgfs.2011.11.003

Fierer, N., and Jackson, R. B. (2006). The diversity and biogeography of soil bacterial communities. *Proc. Natl Acad. Sci. U.S.A.* 103, 626–631. doi: 10.1073/pnas.0507535103

Fornasier, F., Ascher, J., Ceccherini, M. T., Tomat, E., and Pietramellara, G. (2014). A simplified rapid, low-cost and versatile DNA-based assessment of soil microbial biomass. *Ecol. Indic.*, 45, 75–82. doi: 10.1016/j.ecolind.2014.03.028

Fracassetti, D., and Vigentini, I. (2018). "Occurrence and analysis of sulfur compounds in wine," in *Grapes and Wines: Advances in Production, Processing, Analysis and Valorization*, eds A. M. Jordão and F. Cosme (London, UK: IntechOpen) 225. doi: 10.5772/intechopen.72823

Garofalo, C., Russo, P., Beneduce, L., Massa, S., Spano, G., and Capozzi, V. (2016). Non-Saccharomyces biodiversity in wine and the 'microbial terroir': a survey on Nero di Troia wine from the Apulian region, Italy. *Ann. Microbiol.* 66, 143–150. doi: 10.1007/s13213-015-1090-5

Gessa, C., and Ciavatta, C. (2000). "Complesso di scambio," in *Metodi di AnalisiChimicadelSuolo. Ministero per le PoliticheAgricole e Forestali, OsservatorioNazionalePedologico e per la QualitàdelSuolo*, 13, ed F. Angeli (Milano: Franco Ange), 1–31.

Gilbert, J. A., van der Lelie, D., and Zarraonaindia, I. (2014). Microbial terroir for wine grapes. *Proc. Natl. Acad. Sci. U.S.A.* 111, 5–6. doi: 10.1073/pnas.1320471110

Guo, X., Yin, H., Liang, Y., Hu, Q., Zhou, X., Xiao, Y., et al. (2014). Comparative genome analysis reveals metabolic versatility and environmental adaptations of *Sulfobacillus thermosulfidooxidans* strain ST. *PLoS ONE* 9:e99417. doi: 10.1371/journal.pone.0099417

Hammer, Ø., Harper, D. A., and Ryan, P. D. (2001). PAST: paleontological statistics software package for education and data analysis. *Palaeontol. Electron.* 4:9.

He, Z., Deng, Y., Van Nostrand, J. D., Tu, Q., Xu, M., Hemme, C. L., et al. (2010a). GeoChip 3.0 as a high-throughput tool for analyzing microbial community composition, structure and functional activity. *ISME J.* 4:1167. doi: 10.1038/ismej.2010.46

He, Z., Xu, M., Deng, Y., Kang, S., Kellogg, L., Wu, L., et al. (2010b). Metagenomic analysis reveals a marked divergence in the structure of belowground microbial communities at elevated CO_2. *Ecol Lett*, 13, 564–575. doi: 10.1111/j.1461-0248.2010.01453.x

Jahn, R., Blume, H.P., Asio, V.B., Spaargaren, O., and Schad, P. (2006). *Guidelines for Soil Description*. Rome: FAO.

Janosch, C., Remonsellez, F., Sand, W., and Vera, M. (2015). Sulfur oxygenase reductase (Sor) in the moderately thermoacidophilic leaching bacteria: studies in *Sulfobacillusthermosulfidooxidans* and *Acidithiobacilluscaldus*. *Microorganisms* 3, 707–724. doi: 10.3390/microorganisms3040707

Jara, C., Laurie, V. F., Mas, A., and Romero, J. (2016). Microbial terroir in Chilean Valleys: diversity of non-conventional yeast. *Front. Microbiol.* 7:663. doi: 10.3389/fmicb.2016.00663

Kahnert, A., Mirleau, P., Wait, R., and Kertesz, M. A. (2002). The LysR-type regulator SftR is involved in soil survival and sulphate ester metabolism in *Pseudomonas putida*. *Environ. Microbiol.* 4, 225–237. doi: 10.1046/j.1462-2920.2002.00289.x

Kertesz, M. A., Fellows, E., and Schmalenberger, A. (2007). Rhizobacteria and plant sulfur supply. *Adv. Appl. Microbiol.* 62, 235–268. doi: 10.1016/S0065-2164(07)62008-5

Kertesz, M. A., and Mirleau, P. (2004). The role of soil microbes in plant sulphur nutrition. *J. Exp. Bot.* 55, 1939–1945. doi: 10.1093/jxb/erh176

Knight, S., Klaere, S., Fedrizzi, B., and Goddard, M. R. (2015). Regional microbial signatures positively correlate with differential wine phenotypes: evidence for a microbial aspect to terroir. *Sci. Rep.* 5:14233. doi: 10.1038/srep14233

Kritzinger, E. C., Bauer, F. F., and Du Toit, W. J. (2012). Role of glutathione in winemaking: a review. *J. Agric. Food Chem.* 61, 269–277. doi: 10.1021/jf303665z

Kuramae, E. E., Yergeau, E., Wong, L., Pijl, A. S., Van Veen, J. A., and Kowalchuk, G. A. (2012). Soil characteristics more strongly influence soil bacterial communities than land -use type. *FEMS Microbiol. Ecol.* 79, 12–24. doi: 10.1111/j.1574-6941.2011.01192.x

Kuzyakov, Y., and Blagodatskaya, E. (2015). Microbial hotspots and hot moments in soil: concept & review. *Soil Biol. Biochem.* 83, 184–199. doi: 10.1016/j.soilbio.2015.01.025

Lacroux, F., Trégoat, O., van Leeuwen, C., Pons, A., Tominaga, T., Lavigne-Cruège, V., et al. (2008). Effect of foliar nitrogen and sulphur application on aromatic expression of *Vitis vinifera* L. cv. Sauvignon blanc. *OENO One* 42, 125–132. doi: 10.20870/oeno-one.2008.42.3.816

Li, X., Jousset, A., de Boer, W., Carrión, V. J., Zhang, T., Wang, X., et al. (2019). Legacy of land use history determines reprogramming of plant physiology by soil microbiome. *ISME J.* 13, 738. doi: 10.1038/s41396-018-0300-0

Li, Y., He, J., He, Z., Zhou, Y., Yuan, M., Xu, X., et al. (2014). Phylogenetic and functional gene structure shifts of the oral microbiomes in periodontitis patients. *ISME J.* 8, 1879–1891. doi: 10.1038/ismej.2014.28

Loeppert, R. H., and Suarez, D. L. (1996). "Carbonate and gypsum," in *Methods of soil analysis, Part 3: Chemical Methods,* ed D. Sparks (Madison, WI: SSSA and ASA), 437–474. doi: 10.2136/sssabookser5.3.c15

Logan, B. E. (2009). Exoelectrogenic bacteria that power microbial fuel cells. *Nat. Rev. Microbiol.* 7, 375–381. doi: 10.1038/nrmicro2113

Lu, Z., He, Z., Parisi, V. A., Kang, S., Deng, Y., Van Nostrand, J. D., et al. (2012). Geochip-based analysis of microbial functional gene diversity in a land fill leachate-contaminated aquifer. *Environ. Sci. Technol.* 46, 5824–5833. doi: 10.1021/es300478j

Martini, E., Comina, C., Priori, S., and Costantini, E. A. C. (2013). A combined geophysical-pedological approach for precision viticulture in the Chianti hills. *Bollettino Di Geofisica Teorica Ed Applicata*, 54, 165–181. doi: 10.4430/bgta0079

Masepohl, B., Drepper, T., Paschen, A., Gross, S., Pawlowski, A., Raabe, K., et al. (2002). Regulation of nitrogen fixation in the phototrophic purple bacterium *Rhodobacter capsulatus*. *J. Mol. Microbiol.* Biotechnol. 4, 243–248.

Mezzasalma, V., Sand ionigi, A., Bruni, I., Bruno, A., Lovicu, G., Casiraghi, M., et al. (2017). Grape microbiome as a reliable and persistent signature of field origin and environmental conditions in cannonau wine production. *PLoS ONE* 12:e184615. doi: 10.1371/journal.pone.0184615

Miura, T., Sánchez, R., Castañeda, L. E., Godoy, K., and Barbosa, O. (2017). Is microbial terroir related to geographic distance between vineyards?. *Environ. Microbiol. Rep.* 9, 742–749. doi: 10.1111/1758-2229.12589

Mocali, S., Galeffi, C., Perrin, E., Florio, A., Migliore, M., Canganella, F., et al. (2013). Alteration of bacterial communities and organic matter in microbial fuel cells (MFCs) supplied with soil and organic fertilizer. *Appl. Microbiol. Biotechnol.* 97, 1299–1315. doi: 10.1007/s00253-012-3906-6

Nobre, A., Alarico, S., Fernand es, C., Empadinhas, N., and da Costa, M. S. (2008). A unique combination of genetic systems for the synthesis of trehalose in rubrobacter xylanophilus: properties of a rare actinobacterial TreT. *J. Bacteriol.* 190, 7939–7946. doi: 10.1128/JB.01055-08

OIV (2010). *Definition of Viticultural "Terroir". Resolution OIV/Viti333/2010.* Tbilisi: OIV.

Ono, S. I. (1989). Nitrogen mineralization from paddy and upland soils under flooded and non-flooded incubation. *Soil Sci. Plant Nutr.* 35, 417–426. doi: 10.1080/00380768.1989.10434774

Pokorna, D., and Zabranska, J. (2015). Sulfur-oxidizing bacteria in environmental technology. *Biotechnol. Adv.* 33, 1246–1259. doi: 10.1016/j.biotechadv.2015.02.007

Priori, S., Bianconi, N., and Costantini, E. A. (2014). Can γ-radiometrics predict soil textural data and stoniness in different parent materials? A comparison of two machine-learning methods. *Geoderma* 226, 354–364. doi: 10.1016/j.geoderma.2014.03.012

Priori, S., Martini, E., Andrenelli, M. C., Magini, S., Agnelli, A. E., Bucelli, P., et al. (2013). Improving wine quality through harvest zoning and combined use of remote and soil proximal sensing. *Soil Sci. Soc. Am. J.* 77, 1338–1348. doi: 10.2136/sssaj2012.0376

Ravanbakhsh, M., Kowalchuk, G. A., and Jousset, A. (2019). Root-associated microorganisms reprogram plant life history along the growth–stress resistance tradeoff. *ISME J.* 13, 3093–3101. doi: 10.1038/s41396-019-0501-1

Richard, A. H., Qiu, X. Y., Wu, L. Y., Roh, Y., Palumbo, A. V., Tiedje, J. M., et al. (2001). Simultaneous recovery of RNA and DNA from soils and sediments. *Appl. Environ. Microbiol.* 67, 4495–4503. doi: 10.1128/AEM.67.10.4495-4503.2001

Seguin, G. (1986). 'Terroirs' and pedology of wine growing. *Experientia* 42, 861–873. doi: 10.1007/BF01941763

Soubeyrand, E., Basteau, C., Hilbert, G., van Leeuwen, C., Delrot, S., and Gomès, E. (2014). Nitrogen supply affects anthocyanin biosynthetic and regulatory genes in grapevine cv. Cabernet-Sauvignon berries. *Phytochemistry* 103, 38–49. doi: 10.1016/j.phytochem.2014.03.024

Tang, K., Baskaran, V., and Nemati, M. (2009). Bacteria of the sulphur cycle: an overview of microbiology, biokinetics and their role in petroleum and mining industries. *Biochem. Eng. J.* 44, 73–94. doi: 10.1016/j.bej.2008.12.011

Vadakattu, G. V., Bramley, R. G., Greenfield, P., Yu, J., and Herderich, M. (2019). Vineyard soil microbiome composition related to rotundone concentration in Australian cool climate 'peppery'Shiraz grapes. *Front. Microbiol.* 10:1607. doi: 10.3389/fmicb.2019.01607

van Leeuwen, C., Friant,P., Choné X., Tregoat,O., Koundouras, S., and Dubourdieu, D. (2004). Influence of climate, soil and cultivar on terroir. *Am. J. Enol. Vitic.* 55,207–217.

van Leeuwen, C., Roby, J. P., and de Rességuier, L. (2018). Soil-related terroir factors: a review. *OENO one* 52, 173–188. doi: 10.20870/oeno-one.2018.52.2.2208

van Leeuwen,C., and Seguin,G. (2006). The concept of terroir in viticulture. *J. Wine Res.* 17:1–10. doi: 10.1080/09571260600633135

van Nostrand, J. D., Liang, Y., He, Z., Li, G., and Zhou, J. (2010a). Geochip: a high throughput genomic tool for linking community structure to functions. *Handb. Hydrocarbon Lipid Microbiol.* 2677–2685. doi: 10.1007/978-3-540-77587-4_198

van Nostrand, J. D., Wu, L., He, Z., and Zhou, J (2010b). GeoChips for analysis of microbial functional communities. *Handb. Hydrocarbon Lipid Microbiol.* 4039–4050. doi: 10.1007/978-3-540-77587-4_314

Vaudour, E. (2002). The quality of grapes and wine in relation to geography: notions of terroir at various scales, J. *Wine Res.* 13,117–141. doi: 10.1080/0957126022000017981

Vaudour, E., Costantini, E., Jones, G. V., and Mocali, S. (2015). An overview of the recent approaches to terroir functional modelling, footprinting and zoning. *Soil* 1, 287–312. doi: 10.5194/soil-1-287-2015

Wilhelm Scherer, H. (2009). Sulfur in soils. *J. Plant Nutr. Soil Sci.* 172, 326–335. doi: 10.1002/jpln.200900037

Woodruff, P. J., Carlson, B. L., Siridechadilok, B., Pratt, M. R., Senaratne, R. H., Mougous, J. D., et al. (2004). Trehalose is required for growth of *Mycobacterium smegmatis*. *J. Biol. Chem.* 279, 28835–28843. doi: 10.1074/jbc.M313103200

Yan, Y., Klinkhamer, P. G. L., van Veen, J. A., and Kuramae, E. E. (2019). Environmental filtering: a case of bacterial community assembly in soil. *Soil Biol. Biochem.* 136:107531. doi: 10.1016/j.soilbio.2019.107531

Zarraonaindia, I., Owens, S. M., Weisenhorn, P., West, K., Hampton-Marcell, J., Lax, S., et al. (2015). The soil microbiome influences grapevine-associated microbiota. *MBio* 6:e02527-14. doi: 10.1128/mBio.02527-14

Zhang, Y., Li, D., Wang, H., Xiao, Q., and Liu, X. (2006). Molecular diversity of nitrogenfixing bacteria in Tibet Plateau, China. *FEMS Microbiol. Lett.* 260, 134–142. doi: 10.1111/j.1574-6968.2006.00317.x

Zhang, Y., Pohlmann, E. L., and Roberts, G. P. (2005). GlnD is essential for NifA activation, NtrB/NtrC-regulated gene expression, and posttranslational regulation of nitrogenase activity in the photosynthetic, nitrogen-fixing bacterium *Rhodospirillum rubrum*. *J. Bacteriol.* 187, 1254–1265. doi: 10.1128/JB.187.4.1254-1265.2005

Zhou, J., Kang, S., Schadt, C. W., and Garten, C. T. (2008). Spatial scaling of functional gene diversity across various microbial taxa. *Proc. Natl. Acad. Sci. U.S.A.* 105, 7768–7773. doi: 10.1073/pnas.0709016105

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REVIEW
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Defining and Managing for Healthy Vineyard Soils, Intersections With the Concept of Terroir

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The use of sustainable soil management practices is becoming common in wine growing regions around the world in response to an increased awareness of the value of soil health to maintain environmental quality, crop yield, and grape quality. In spite of this, little information is available on the meaning of soil health within a viticultural context, the effects of soil management practices on soil health and the consequences for grape quality and the expression of the terroir. In this review we discuss interrelated dynamic physical, chemical, and biological properties associated with soil health and how they could be important in the expression of the terroir. We focus on the use of cover crops and compost application, two practices commonly used in vineyard soils, and how they affect these physical, chemical and biological aspects of soil health, grape quality and the expression of the terroir. Finally, we discuss research gaps, and best management practices to reduce possible tradeoffs associated with these practices such as the emission of greenhouse gasses.

Keywords: sustainable soil management, viticulture, C sequestration, compost, cover crops, soil quality

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INTRODUCTION

Increasing awareness that soils are a non-renewable resource and the prevailing role of soils in climate change mitigation have spurred worldwide efforts to protect and improve soil health. Recent estimates indicate that 36 billion tons of soil are lost annually due to water and wind erosion alone (Borrelli et al., 2017). Besides erosion, soils are threatened by soil organic matter (SOM) loss, soil nutrient imbalances, salinization and sodification, soil sealing and land intake, loss of soil biodiversity, contamination, acidification, compaction, and waterlogging (FAO and ITPS, 2015). The term "Soil Health" has been co-opted by governmental and non-governmental organizations, academic institutions, industry and grassroot organizations alike in an effort to raise awareness of soil conservation and protect soils from degradation (Karlen et al., 2019). This urgency to protect soils has been further strengthened by their potential to store carbon (C) that would otherwise be emitted into the atmosphere as carbon dioxide (CO_2), thereby offering an appealing pathway for climate change mitigation (Lal, 2004; Paustian et al., 2016). France announced the four Permille Initiative at the Climate Summit in Paris in 2015, aimed at reducing greenhouse gas (GHG) emissions and increasing food security by increasing soil organic carbon (SOC) concentrations by four permille worldwide (Minasny et al., 2017). Similar initiatives have followed, including California's Healthy Soils Program which was launched in 2017 (Ross, 2016).

Conservation of soils and soil ecosystem services has been proposed as a key strategy to attain the United Nations Sustainable Development Goals by 2030 (Keesstra et al., 2016, 2018), and implemented in the European Union through payment for ecosystem services in rural development programs (Galati et al., 2016).

Under these initiatives, management practices that increase sequestration of SOC are promoted or incentivized. Much of the research on soil health and C sequestration have focused on cereal cropping systems in temperate climate zones (Fine et al., 2017). Given important differences between vineyards and cereal cropping systems, it is pertinent to evaluate soil health in the context of vineyard soils in particular and discuss the implications for the winemaking industry.

Vineyards in the old world were often established on marginal land, reserving the most fertile soils for cultivation of cereals, and other food crops (Martínez-Casasnovas and Ramos, 2006; Blavet et al., 2009), while extensive availability of land in the new world offered deep, alluvial, fertile soils for vineyard establishment (White, 2015). As a result, vineyards arguably occupy a broader range of soil types than any other crop. Due to microclimatic effects on winegrape quality, vineyards are often established on slopes, taking advantage of the hill's aspect to optimize solar radiation for the crop (White, 2015). Vineyards on slopes and marginal lands especially are at elevated risk for soil degradation, causing declines in soil quality and fertility, reduced water infiltration and storage, impaired quality of ground and surface water, diminished air quality, and risks associated with climate change mitigation and adaptation. Meanwhile, vineyards have a relatively low nitrogen (N) requirement, implying a low risk for N pollution associated with N fertilization compared to other crops (Williams, 1999). Moreover, as perennial crops with a relatively deep root system, vineyard soils were suggested to have great potential to sequester C (Suddick et al., 2013). Viticulture practices often aim to optimize wine grape quality rather than yield, as winemakers seek to bring out the flavors of a specific terroir, while consumers increasingly value sustainable practices in their decision process when buying wines (Schäufele and Hamm, 2017). As such, many questions remain on how to balance soil management to optimize soil health and the expression of terroir. Therefore, the objectives of this review are to (1) explore where soil characteristics for soil health and fine wine terroir intersect; (2) synthesize the literature on how cover crops and compost application affect soil health and terroir in vineyards; and (3) identify knowledge gaps in the management of cover crops, compost application and other novel practices to improve soil health and terroir.

WHERE SOIL HEALTH MANAGEMENT AND THE EXPRESSION OF TERROIR INTERSECT

The concept of soil health or soil quality has been of concern to soil scientists and agronomists for decades. Various attempts have been made to define soil health and identify metrics and frameworks to quantify the health status of a soil (Karlen and Rice, 2015; Bünemann et al., 2018; Stewart et al., 2018). The United States Natural Resources Conservation Services (NRCS) defined soil health as "the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals, and humans," an adaptation of the earlier definition of soil quality by Doran (1994). A key component in this definition is the importance of soil functions; a healthy soil does not only show certain properties, these properties link to the capacity of the soil to perform functions or deliver ecosystem services that benefit humankind (Doran, 1994; Haygarth and Ritz, 2009). In this review, we adopted previously proposed concepts of soil functions (Schulte et al., 2014; Vogel et al., 2019), and envision a healthy soil to (1) support high yield and crop quality, (2) have good water infiltration and the capacity to store water, (3) retain and recycle nutrients efficiently, while supplying nutrients in correspondence with crop demand, (4) store C and reduce GHG emissions, and (5) support diverse microorganisms and high biological activity (**Figure 1**). The capacity of a soil to perform these soil functions can be assessed by a suite of chemical, physical, and biological soil health indicators; however, a standardized approach to assess soil health remains elusive (Fine et al., 2017; Stewart et al., 2018; Vogel et al., 2019).

For the objective of winemaking, the most important soil function to be optimized is support of high crop quality. It is well known that soils influence the quality of winegrapes; in fact, the impact of the soil, when combined with climate, topography and grapevine variety, is referred to as the terroir effect (Van Leeuwen et al., 2018). When we consider the soil component of the terroir effect in relation to soil health, it is helpful to distinguish (i) inherent (or use-invariant) soil properties from (ii) dynamic or manageable soil properties (Wienhold et al., 2004; Karlen et al., 2019). The soil component of terroir has been linked to factors such as soil depth and minerology (White, 2015), which would be considered inherent soil properties, while other studies have found effects of soil nutrient supply, soil moisture, and soil temperature on winegrape quality (Van Leeuwen et al., 2018), which are considered dynamic soil properties. In this review, we deem that soil health is reigned by dynamic soil properties, and that a healthy soil is a soil that performs soil functions to its maximum capacity. This is in line with Fine et al. (2017), who proposed scoring schemes for soil health indicators based on soil texture groups, as implemented in Cornell's comprehensive soil health assessment. It also resonates with Vogel et al. (2019), who considered that each soil has a limited potential to deliver a certain soil function, and that soil health assessment should reflect the status of a soil relative to its potential. As such, soil health and terroir are expected to intersect with respect to the dynamic soil components of terroir (**Figure 2**). Because crop quality is one of the functions performed by a healthy soil, we postulate that a healthy vineyard soil must optimally express its terroir. While the concept of terroir and its relationship with crop quality is well understood and deeply engrained in the context of wine production, the concept of soil health has recently gained acceptance by growers worldwide (Brevik et al., 2019) and there is an urgent need to reconcile these two concepts. Here, we discuss how dynamic soil properties of terroir intersect with soil health.

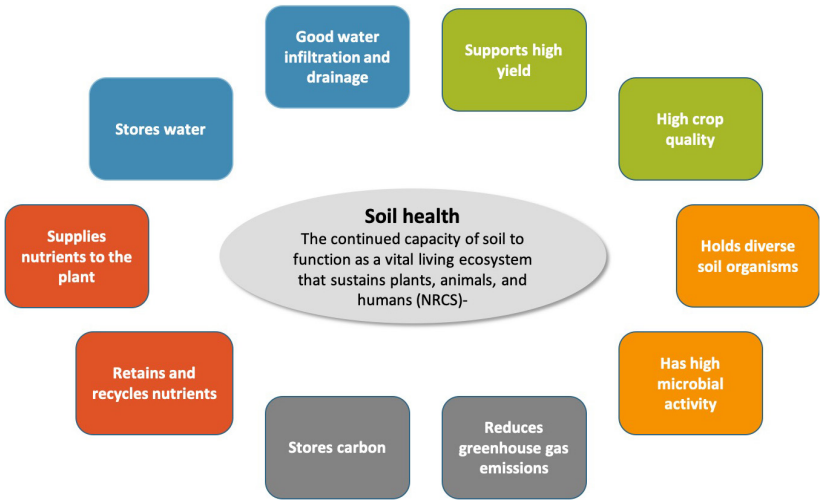

FIGURE 1 | Definition of soil health with representation of soil functions performed by a healthy soil.

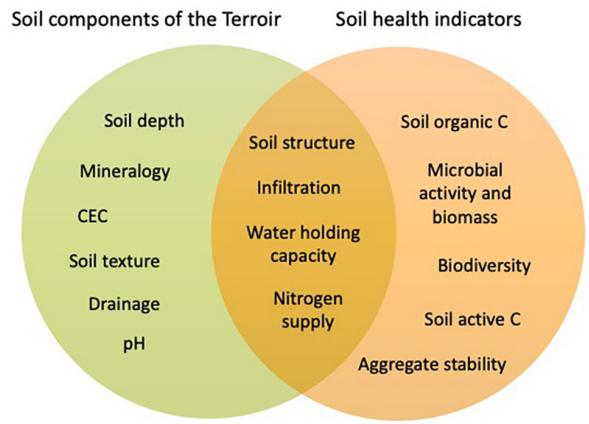

FIGURE 2 | Venn diagram illustrating where soil health and terroir intersect.

Soil Temperature

Van Leeuwen et al. (2018) proposed that cool soils may be an advantage in warm climates because they can slightly delay ripeness, thereby enhancing certain flavors. Soil temperature is greatly affected by soil color; light colors have a high albedo effect which keeps the soil relatively cool. Soil color can be described as an inherent soil property. However, light colors on the soil surface can be achieved by management practices such as mulching and leaving cover crop residue on the surface (Teasdale and Mohler, 1993). While soil temperature appears relevant to terroir, it has not been commonly associated with soil health assessment. Management practices that decrease soil temperature, however, are greatly promoted in regard to other soil health functions.

Soil Chemical Fertility

It can be argued that soils of the best terroirs should be characterized by a stable and well balanced nutrient supply, able to assure the target qualitative result without massive integration of fertilizers (Costantini and Bucelli, 2014). Experience shows that these soils are often characterized by only moderate chemical fertility. Correlations between the mineral composition in soils and wine has been used with mixed success to identify the geographic origin of wines (Versari et al., 2014; Pepi et al., 2017). Yet, scientific evidence linking soil chemical properties to grape and wine quality is scarce. Maltman (2013) argues that the minerals in wine are typically metallic cations at minuscule concentrations that lack flavor, and only distantly relate to vineyard geological minerals. Meanwhile, plant nutrients play an important role in plant physiology and metabolism. Mackenzie and Christy (2005) found that grape juice properties such as Baumé and titratable acidity (TA) were correlated with several plant-available trace elements in the soil, most notably Ca, Sr, Ba, Pb, and Si. Potassium content of soil has been found to have an effect on must acidity (Costantini and Bucelli, 2014), and manganese has been correlated with phenolics in grape berries (Bramley and Janik, 2005). While mechanisms underlying these correlations are lacking, the results suggest that soil cation chemistry does have an influence on wine grape composition. We increasingly understand how soil biological processes govern the availability of plant essential and beneficial nutrients and acknowledge that soil management has the potential to modify these processes. In particular, increased popularity of organic amendments such as composts in vineyards could greatly affect the availability of essential and beneficial plant nutrients. Hence, it likely behooves viticulturalists to better understand how managing soil nutrient supply can optimize the soil chemistry related expression of terroir.

Soil Nitrogen Supply and Retention

Nitrogen is one of the most limiting nutrients for plant growth, and by far the dominant nutrient applied as fertilizer to cropping systems worldwide. As ensuring high N supply is considered a

type of insurance for high crop production, it is not surprising that average N use efficiencies (i.e., the amount of applied N taken up by the crop) are as low as 50% (Ladha et al., 2005). N not taken up by the plant is commonly lost from the soil-plant system through leaching or gaseous emission (**Figure 3A**). While an adequate supply of N is crucial for good crop productivity, the overapplication of N fertilizer over the last decades has caused large losses of N to the environment with alarming consequences for air and water resources (Rockström et al., 2009).

Soil NO_3^- concentrations are typically shown on soil test reports and have been used to monitor the availability of N for the plant. Good nutrient stewardship keeps soil NO_3^- concentrations high enough to meet crop N demand, and low enough to reduce the risk of N loss to the environment. However, improved understanding of the N cycle increasingly demonstrates that NO_3^- is not a static pool waiting to be taken up by the plant. Instead, soil NH_4^+ and NO_3^- pools can be very dynamic, reliant on rapid mineralization, nitrification and immobilization rates that continuously produce and consume new plant available N (Drinkwater and Snapp, 2007; Bowles et al., 2015). In this scenario, plants successfully compete with microorganisms for the use of newly mineralized plant available N, and soil NO_3^- concentrations can likely be kept much lower than previously thought, further reducing N losses to the environment (**Figure 3B**). Following this paradigm shift, a healthy soil supplies N when the plant needs it and retains N during periods of low plant demand by cycling it efficiently through a thriving soil food web.

In most cropping systems, the success of this new paradigm is subject to the ability of the plant to compete with microorganisms for soil available N. In wine grape production, however, the story is even more complicated. As for most crops, N plays a major role in many biological functions and processes and is therefore a highly abundant and often limiting nutrient in grapevine production. Winegrapes remove about 22–56 kg of N ha^{-1} through harvest (Williams, 1999), and this value serves as an indication of the minimum amount of N that should be replenished annually. In addition, a great amount of N is invested in the growth of trunk, roots, leaves and shoots. Although a fraction of this N is reabsorbed into permanent structures when plants go dormant or recycled in the soil-plant system with leaf fall and pruning, vegetative growth does imply additional N demand. Meanwhile, excessive N availability can boost vigor, negatively impacting sink-source relationships in the vine and the canopy microclimate, all of which can lead to loss of grape yield and quality (Wheeler and Pickering, 2003; White et al., 2007). Moreover, N nutrition also directly affects the form and concentration of yeast assimilable nitrogen (YAN) in the grapes, with further implications for wine quality (Bell and Henschke, 2005; Reynard et al., 2011). It is clear that to balance vine health and grape quality, vineyard N status must be managed very carefully.

Traditionally, excessive availability of N in vineyards is avoided by delivering N in small doses when vine N demand is greatest (**Figure 3C**). When building SOM and soil health, however, care must be taken that this does not inadvertently lead to an increase in the soil's capacity to supply N, thereby promoting vine vigor and loss of wine grape quality. As the balance between N mineralization and immobilization is governed to a great extent by the availability of C relative to N (Booth et al., 2005), C-rich organic inputs such as straw amendments have been proposed as one strategy to manage excessive N supply in healthy soils (Wheeler and Pickering, 2003) (**Figure 3D**). Alternatively, non-legume cover crops have been used to remove excessive soil NO_3^-, by increasing competition with the vines for available N (Wheeler and Pickering, 2003; Van Leeuwen et al., 2018).

Water Availability and Drainage

In wine grape cultivation, it is important to carefully manage water availability and drainage in order to ensure vine health as well as wine grape quality. Excessive water availability can promote vigor and cause direct and indirect negative effects on wine grape quality (Wheeler and Pickering, 2003; White et al., 2007). Therefore, high wine grape quality is often expressed under mild water stress, as found on well-drained soils or as a result of deficit irrigation. If water stress becomes too severe, however, vine health, yield as well as wine grape quality are jeopardized. This is particularly relevant for vineyards in Mediterranean climates where water scarcity can become an issue, especially in the light of climate change (Medrano et al., 2015; Mirás-Avalos et al., 2017).

Whether a vineyard experiences excessive water supply or severe water stress depends on the climate, the cultivar, the rootstock, irrigation management, canopy management, soil type, as well as soil health management (Medrano et al., 2015). Various soil properties affect soil water regulation, most notoriously water holding capacity, plant available water, and hydraulic conductivity. Water holding capacity is the water the soil can hold on to against gravitational forces. The water holding capacity reflects the amount of water in the soil at a matric potential of approximately $^-$33 kPa. Permanent wilting point is reached at a matric potential of approximately $^-$1,500 kPa. At this point, water left in the soil is strongly held by capillary forces and unavailable to the plant. Therefore, plant available water is defined as the amount of water observed between field capacity and permanent wilting point. Finer textured soils have a greater water holding capacity compared to coarser textured soils (**Figure 4**). Meanwhile, the greatest plant available water is typically observed in silt loams and silty clay loams. In finer soil texture classes, plant available water decreases as the water left in the soil at the permanent wilting point increases. Even though water holding capacity and plant available water are predominately determined by soil texture class, building soil health by increasing SOM and improving soil structure has the capacity to increase water holding capacity and plant available water (Kern, 1995; Olness and Archer, 2005). While water holding capacity and plant available water are important metrics for water storage, hydraulic conductivity describes the ease with which water can move through the soil. Hydraulic conductivity is greatly affected by texture and soil moisture. Hydraulic conductivity is typically greater in coarse textured

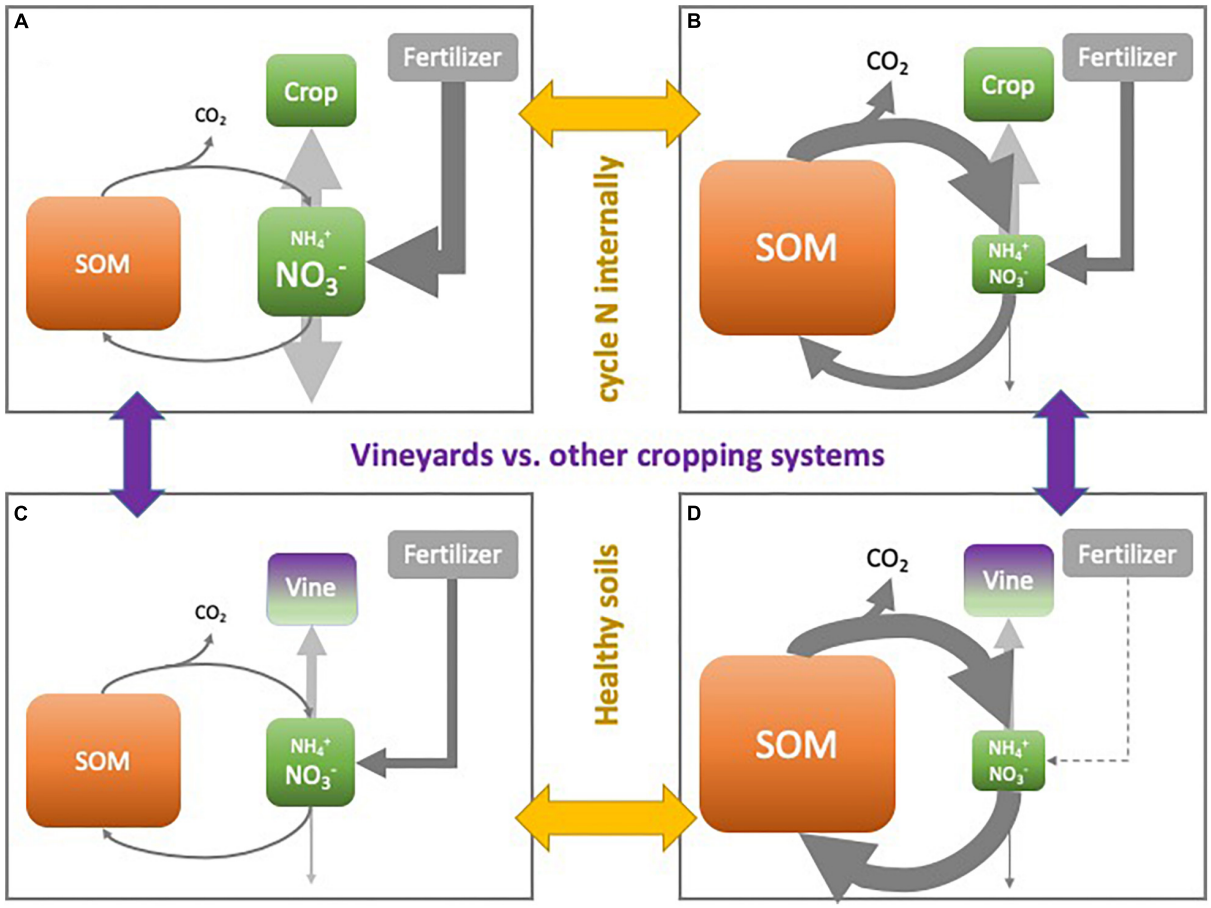

FIGURE 3 | Illustration of the N cycle for **(A)** conventional agricultural systems for crops with high N demand, **(B)** agricultural systems for crops with high N demand under soil health management, **(C)** conventional vineyard system, and **(D)** vineyards with soil health management.

soils under very wet conditions but as the soil becomes dryer it decreases sharply and, at low soil moisture conditions, hydraulic conductivity is lower than in fine textured soils (**Figure 4**). In addition to the inherent property soil texture, manageable soil properties including SOM content and soil structure have been shown to affect hydraulic conductivity (Vereecken, 1995).

From a soils water balance perspective, soil water supply is determined by infiltration rate, storage, drainage, runoff, and evapotranspiration (**Figure 5**). In a fine-textured soil, the amount of water stored in the soil may be compromised if runoff is high relative to infiltration. In contrast, water supply may be excessive in fine textured soils in wetter climates, due to the high water holding capacity of fine textured soils (Van Leeuwen et al., 2018). Soil health management can be tailored to improve infiltration and water storage in dry climates; or mitigate excessive water storage in fine textured soils by improving hydraulic conductivity and drainage or promoting evapotranspiration in wetter climates. Meanwhile, the low water holding capacity of coarse textured soils causes increased risk for severe water stress in dry climates. Here, soil health management can increase water holding capacity and help mitigate severe water stress (Medrano et al., 2015). As such, soil health management regarding water regulation can greatly impact the terroir effect.

Soil Biodiversity

There is a growing interest in the role of the soil microbiome not only for grapevine health and nutrition but also for wine quality. The soil microbiome includes all microorganisms that can be found in soil, including archaea, bacteria, viruses, fungi, protists, and other microbial eukaryotes (Fierer, 2017). For most of the history of viticulture, and agriculture in general, soil microbes were mostly seen as something negative (i.e., pathogens), and efforts to improve wine quality were mostly focused on grapevine genetics and soil cultural practices, with little consideration for the soil and plant microbiome. The increasing availability, affordability and use of molecular techniques is revealing the existence of a great amount of biodiversity in the soil, the root–soil interface (the rhizosphere) and the plant itself with beneficial functions for plants (Vandenkoornhuyse et al., 2015). Soil microbial communities support key ecological processes and are directly responsible for the provision of the most important soil ecosystem services such as decomposition, mineralization of

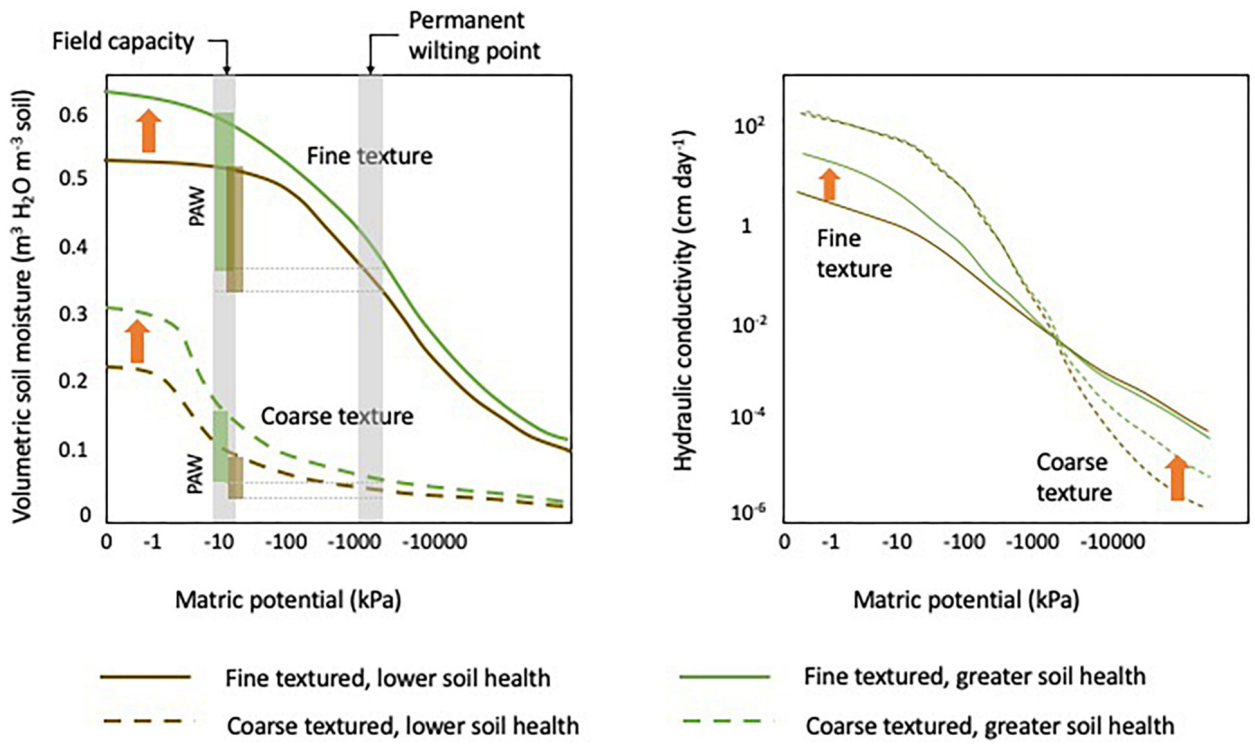

FIGURE 4 | Left: Conceptual representation of soil moisture retention curves for coarse textured soils (dashed line) and fine textured soils (solid lines) soils with lower soil health (brown lines) compared to soils with greater soil health (green lines). Columns represent the amount of plant available water (PAW). Right: Conceptual representation of unsaturated hydraulic conductivity in function of soil matric potential for coarse textured soils (dashed line) and fine textured soils (solid lines) soils with lower soil health (brown lines) compared to soils with greater soil health (green lines). Orange arrows in both figures indicate the direction of improved soil health. Charts modified from Brady and Weil (2016).

plant nutrients, atmospheric N fixation and C sequestration, all of them of high relevance for agricultural production (Bardgett and van der Putten, 2014; Fierer, 2017). Plants with adequate nutrition may be more resistant to biotic stress; in addition, soil microorganisms are known to support plant health by competing with pathogenic microorganisms for soil resources, production of antibiotic substances, or triggering of plant defenses, the so-called induced systemic resistance (Berendsen et al., 2012). But the role of the soil microbiome in wine production goes beyond supporting the typical functions of a healthy soil, wine making is essentially a microbial process in which the whole grape microbiome in early fermentation stage, including both bacteria and fungi, drives fermentation performance and the chemical characteristics of the finished wine (Bokulich et al., 2013; Belda et al., 2017). The microbial community of the winegrapes is known to share several microbial taxa with the soil, which suggests that soil may serve as a reservoir for grape microorganisms that are carried over into the fermentation stage (Zarraonaindia et al., 2015). Soil microorganisms may colonize the grapes through different pathways, such as the migration of root endophytes, dust, rain splashes, or people during harvesting and other management events (Gilbert et al., 2014). Therefore both the soil and grape microbiome are as important as the grape varietal, the soil type or the climate in regulating the organoleptic properties of wine, and show a strong biogeographical pattern that allows to differentiate between wine growing regions and even vineyards (Bokulich et al., 2016; Belda et al., 2017). This has led several scientists to propose the concept of the *microbial terroir* (Belda et al., 2017) opening a new and promising research field in which the soil and plant microbiome could be manipulated to support plant health, fermentation and produce optimal organoleptic wine properties. This would be achieved through management for a healthy soil microbiome as an integral part of the soil ecosystem to achieve the maximum expression of the terroir. Recent evidence shows that practices such as cover cropping results in significant changes in the soil microbiome, potentially altering the grape microbiome and wine quality (Burns et al., 2016). Yet, Chou et al. (2018) found little change in the grape microbiome with soil management practices such as herbicide application or cultivation for vegetation removal under the vine.

Soil Carbon Sequestration and Greenhouse Gas Emissions

Soil C sequestration has been promoted as a powerful strategy to mitigate climate change (Lal, 2004). In most soils, the predominant form of C is organic C. Soils serve as a sink for atmospheric CO_2 when management practices that increase SOC stocks are adopted. Soil C sequestration hinges on the balance

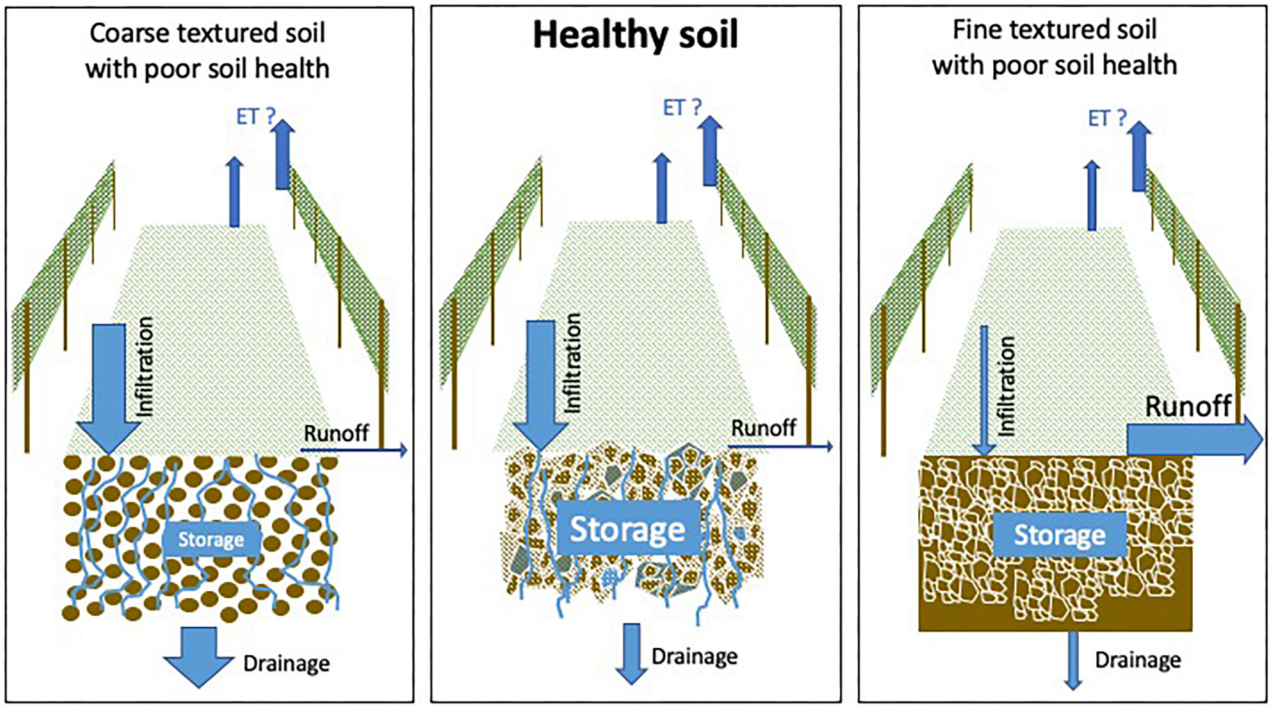

FIGURE 5 | Vineyard water fluxes from a soil perspective in coarse textured soil with poor soil health (left), a healthy soil (middle), and a fine textured soil with poor soil health (right).

between C inputs and losses from decomposition. Soil C inputs can be increased through organic amendments or cover crops, but the net effect on SOC stocks depends on how much of the C that enters the soil is ultimately retained. Relatively stable SOC pools are typically thought to be chemically protected (binding with clay and silt minerals), physical protected (occlusion into soil aggregates), or biochemically stabilized (condensation of recalcitrant C compounds) (Six et al., 2000). Moreover, rather than a pool of inherently stable and chemically unique compounds, evidence has mounted that SOM is a continuum of progressively decomposing organic compounds processed through the soil food web (Lehmann and Kleber, 2015; Kögel-Knabner, 2017). Therefore, C sequestration requires continuous management of the turnover and volume of organic compounds and warrants further research into balancing both stocks and flows of organic matter (Lehmann and Kleber, 2015).

When building SOC stocks as a means to mitigate climate change, one must also consider tradeoffs from GHG emissions, most notably the potent GHG nitrous oxide (N_2O). N_2O is produced predominately by nitrifying and denitrifying microorganisms in the soil and stimulated by anthropogenic and natural disturbances such as fertilizer and organic amendment inputs, rain events, irrigation, and tillage (Butterbach-Bahl et al., 2013; Verhoeven et al., 2017). While building SOC stocks is a slow process that may take 5–10 years before a measurable management impacts are observed, responses of N_2O emissions to management changes are much more instantaneous. The net effect of management on GHG emission reductions takes the balance between changes in SOC stocks and changes in N_2O emissions over multiple years.

Several studies assessing the C balance of vineyards have found that vineyards have great potential for storing C (Kroodsma and Field, 2006; Suddick et al., 2013; Brunori et al., 2016; Scandellari et al., 2016). Kroodsma and Field (2006) estimated that 68 g C m^{-2} $year^{-1}$ could be sequestered by converting annual cropping systems to vineyard agroecosystems in California. Nevertheless, the potential of vineyards to sequester C depends on soil physical characteristics, the grapevine's biological properties, as well as management practices in the vineyard (Brunori et al., 2016; Vicente-Vicente et al., 2016). Most measurements of N_2O emissions in vineyards have taken place in Mediterranean climates. Under these environmental conditions, N_2O emissions in vineyards are relatively small compared to potential to sequester SOC (Garland et al., 2011; Longbottom and Petrie, 2015). Nevertheless, N_2O emissions from vineyards can be further reduced by modifying the timing of N fertilizer application, offsetting soil and cover-cropping activities in the tractor row in relation to forecasted precipitation and minimizing floor management activity in the fall is also recommended (Longbottom and Petrie, 2015; Verhoeven et al., 2017).

What does this all mean for terroir? Soil C sequestration and GHG emissions will unlikely have a direct effect on terroir. However, it is well-known that SOC is essential for maintaining a good soil structure, retaining nutrients, and supporting an active soil food web, all of which have been linked to terroir. Moreover, market research shows that wine

consumers increasingly value sustainability in wine production, especially among higher spenders and wine experts (Pomarici, 2016; Schäufele and Hamm, 2017). In a recent review of articles published between 2000 and March 2016 (Schäufele and Hamm, 2017) found that a considerable number of consumers across different countries reported a willingness to pay a premium for wine with characteristics of sustainable production and that sustainability cues were often perceived as quality indicators.

As a next step, efforts should be focused on establishing the spatial variability and mapping of soil C and soil biodiversity as components of soil and the terroir (Vaudour et al., 2015). Furthermore, it is important to understand how soil management commonly used to support soil health in wine grape production, will affect the expression of the terroir.

COVER CROPS AND COVER CROP MANAGEMENT FOR IMPROVED VINEYARD SOIL HEALTH AND EXPRESSION OF TERROIR

Cover crops are traditionally used in the vineyard interrow to prevent soil degradation and erosion (Battany and Grismer, 2000; Ruiz-Colmenero et al., 2013; Novara et al., 2018), a practice that is being increasingly incentivized in most of the wine growing regions of the world such as California or the Mediterranean countries of Europe, where soil degradation is a pressing issue (Rodrigo-Comino, 2018; Rodrigo-Comino et al., 2018). Moreover, cover crops provide multiple other services, depending on the plant species used, such as acting as catch crops, fodder or green manure (Ramírez-García et al., 2015). Additionally, several recent meta-analyses show that cover crops support ecosystem services such as above- and below-ground biodiversity, pest control, C sequestration and soil fertility (Vicente-Vicente et al., 2016; Bowles et al., 2017; Winter et al., 2018; Shackelford et al., 2019). Many of the ecosystem services provided by cover crops are mediated through effects on soil physical, chemical and biological properties that support soil health and that may affect the expression of the terroir.

Even though the use of cover crops to manage soil fertility dates back to the Roman empire, this practice was mostly abandoned after the green revolution and widespread availability of synthetic fertilizers (Dunn et al., 2016). Today, in spite of the increasing evidence of the benefits for soil health, adoption rates are still very low due to the contrasting perceptions of producers on the actual benefits provided by cover crops, concerns over water and nutrient usage (especially in arid regions), and lack of adequate management strategies (Dunn et al., 2016; Schütte and Bergmann, 2019). Furthermore, cover crops can act as vectors or increase susceptibility of vines to plant diseases (Forte et al., 2010; Muscas et al., 2017). These issues limit the adoption of this practice and drive the active removal of vegetation in the vineyard interrow through the use of tillage and/or herbicides with negative consequences for soil health. As consumers demand sustainable products and are willing to pay the premium of sustainably grown wine, air and water quality regulations are imposed, growers are forced to consider the use of cover crops. Thus, there is a strong need for a careful synthesis of the potential benefits of this practice and how management decisions can reduce potential drawbacks.

Cover crops protect the soil from the eroding action of raindrops preventing soil erosion and runoff (Battany and Grismer, 2000), but they also improve infiltration rates (Gulick et al., 1994; Biddoccu et al., 2017) which is particularly important for vineyards established on steep slopes with higher erosion rates (Ruiz-Colmenero et al., 2013; Rodrigo-Comino, 2018). Higher infiltration, lower runoff and erosion rates in cover cropped vineyards lead to lower nitrate runoff and therefore the increase in nutrient retention (García-Díaz et al., 2017). Higher infiltration has been attributed to the increase in SOM, improvement of structure, including aggregate stability and pore connectivity (Aljibury and Christensen, 1972; Ruiz-Colmenero et al., 2013). Cover crop termination by mowing increases infiltration rates by 45% as compared to tillage (Ruiz-Colmenero et al., 2013). It is also well known that vineyards under cover crops and with a better soil structure store more water than soils without permanent vegetation cover or tilled (García-Díaz et al., 2017; López-Vicente and Álvarez, 2018).

Improvements in soil structure, pore connectivity and water holding capacity are interrelated soil properties which are directly affected by the growth of plant roots and input of organic matter not only through aboveground biomass incorporation but mostly through root turnover and root exudates released from living roots (Sokol et al., 2019). More than half of the plant biomass is belowground, which constitutes a large input of C directly into the soil; the release of root exudates constitutes a constant drip of C that feeds the rhizosphere microbial community leading to the formation of stable C-mineral associations, which contribute to further aggregate formation (Sokol and Bradford, 2019). In Mediterranean climates cover crop growth occurs during the wet season (fall and winter) and afterward, during the dry season, annual cover crops die down whereas perennial plants go into dormancy. Even though this stops root exudation, the role of roots in maintaining root structure during the dry season is still highly relevant, as dead roots hold together and preserve microbially created macroaggregates (Blankinship et al., 2016). As a result, total soil organic C and aggregate stability increases in vineyard soils with cover crops as compared to bare soil (Guzmán et al., 2019); these increases are more pronounced in the upper centimeters of soil (Wolff et al., 2018) and seem to depend on cover crop management; for instance, certain cover crop mixes result in higher C sequestration rates and increases in soil C and improvements in soil structure are higher if cover crop is not tilled (Winter et al., 2018; Novara et al., 2019).

Above and belowground C inputs by cover crops influence the structure of the soil microbial community, increasing microbial biomass and changing the species composition. Burns et al. (2016) observed that cover crop presence and species drove soil microbial community composition and consistently resulted in distinctive bacterial and archaeal soil communities throughout 19 vineyards in Napa Valley, California. As mentioned earlier, the soil microbiome has important implications, not only for nutrient cycling and soil health in general, but also for the health, yield

and quality of the grapevine, and it is currently regarded as an important component of the terroir (Belda et al., 2017).

In addition to changes in the structure of the soil microbial community, cover crop C inputs increase microbial activity, soil respiration and CO_2 efflux as compared to non-cover cropped and tilled vineyard soils (Steenwerth and Belina, 2008b). N inputs with leguminous cover crops could increase the amount of available N in the soil and therefore nitrification and denitrification rates that lead to the production and release of N_2O, a potent GHG (Garland et al., 2011). Increase in CO_2 and N_2O emissions to the atmosphere could offset the potential environmental benefits of cover crops. Nevertheless, the amount of total C sequestered in plant biomass and the increase in soil C usually exceeds the increased CO_2 emissions under cover crops (Wolff et al., 2018). Emissions of N_2O from vineyard soils are usually small but can be increased by soil management practices that affect soil nutrient content, labile C and moisture (Verhoeven et al., 2019). Reductions in N_2O emissions from cover cropped soils could be potentially achieved through the selection of the cover crop mix to reduce the presence of legumes or the choice of cover crop termination method (tilling, mowing, or grazing), however, no clear differences have been observed yet in this respect (Garland et al., 2011; Wolff et al., 2018).

Changes in soil microbiome, physical and chemical properties could have significant impacts to vine vegetative growth, yields, grape and must quality, by changing water and nutrient availability. Cover crops can reduce N uptake during grapevine vegetative growth and reduce vigor and N nutritional status, increasing the anthocyanin and polyphenols contents in the grapes and therefore having a positive impact in must quality (Pérez-Álvarez et al., 2013).

Nevertheless, cover crops can also be large sources of plant-available N if the mix contains legumes (Novara et al., 2019). In addition to improving soil nutrient status, potential nitrification, N mineralization and denitrification can be up to 2–4-fold greater in cover crop soils than in bare soils under tillage showing increased nutrient turnover and availability (Steenwerth and Belina, 2008a). Higher N availability under legume cover crops results in larger vine vegetative growth and reduced polyphenol in grapes as compared to non-legume cover crops (Muscas et al., 2017).

Finally, high transpiration rates in the cover crop can reduce water availability for grapevines; while this may be convenient in humid regions to regulate growth, reduce vigor and avoid negative impacts to must quality, in Mediterranean arid climates it may induce water stress in the grapevine (Celette et al., 2008; Celette and Gary, 2013). Water stress can have beneficial effects for grapevine health and must quality when it is not excessive, as it reduces vegetative growth and increases anthocyanins and polyphenol contents in the grapes (Monteiro and Lopes, 2007).

In spite of the above, several studies have seen little differences between cover cropped and non-cover cropped grapevines, suggesting that this practice does not seem to negatively affect crop vine yield and quality overall (Sweet and Schreiner, 2010; Steenwerth et al., 2013; Pérez-Bermúdez et al., 2016; Winter et al., 2018; Wolff et al., 2018), and that negative effects can be reduced by management decisions. For instance, no tilling the cover crops can reduce grapevine N uptake as compared to tilling (Steenwerth et al., 2013). Fertilizer and irrigation management, mowing the cover crop at budbreak or reducing the sowing density can reduce competition between grapevines and cover crops in dryer and warmer growing regions (Tesic et al., 2007; Delpuech and Metay, 2018). Younger vines may also be more sensitive to water and nutrient stress in the presence of cover crops, as they don't have a sufficiently developed root system to explore different parts of the soil profile or store nutrients (Celette et al., 2008).

COMPOST USE AND MANAGEMENT FOR IMPROVED VINEYARD SOIL HEALTH AND EXPRESSION OF TERROIR

One widely adopted method to improve soil health in vineyards is through the application of composts or other organic input materials (Morlat and Chaussod, 2008; Brown and Cotton, 2011; Gaiotti et al., 2017). Increases in SOC and SOM from compost addition underlie most of the biological, chemical, and physical impacts to vineyard soils we will review in the following sections. SOC or SOM are universally increased from compost additions in vineyard soils (Pinamonti, 1998; Korboulewsky et al., 2002; Ramos and López-Acevedo, 2004; Morlat and Chaussod, 2008; Brown and Cotton, 2011; Bustamante et al., 2011; Peregrina et al., 2012; Rubio et al., 2013; Calleja-Cervantes et al., 2015a,b; Gaiotti et al., 2017; Mondini et al., 2018). Higher rates of compost addition typically result in greater treatment effects between compost treated soils and controls or grower standard NPK fertilizer practices (Korboulewsky et al., 2002; Morlat and Chaussod, 2008; Peregrina et al., 2012; Mondini et al., 2018). Both short term applications (Larchevêque et al., 2006; Rubio et al., 2013) and long-term applications (Morlat and Chaussod, 2008; Calleja-Cervantes et al., 2015a,b) result in increases in SOC/SOM in compost treated plots. The amount of SOM increases linearly with the amount of C applied in composts (Mondini et al., 2018). The longer the continued application, the stronger the treatment effect with time (Morlat and Chaussod, 2008). Soil C is the basis of the soil food web and increases in SOC from compost addition typically increases the size of the microbial biomass (Bustamante et al., 2011; Rubio et al., 2013; Wilson et al., 2016), although not always (Gaiotti et al., 2017). Increases in SOM lead to increases in total N, absolute amounts of organic N as well as inorganic N from mineralization (Larchevêque et al., 2006; Morlat and Chaussod, 2008; Calleja-Cervantes et al., 2015a; Gaiotti et al., 2017). Additions of compost derived C can influence P dynamics through competitive inhibition of P sorption sites by organic acid anions, as well as providing a source of mineral and organic P (Hue, 1992; Korboulewsky et al., 2002; Hunt et al., 2007; Wilson et al., 2016). Increases in SOC lead to better soil aggregation, infiltration and water holding capacity and reduced bulk density (Celik et al., 2004; Morlat and Chaussod, 2008; Brown and Cotton, 2011; Salomé et al., 2016; Ramos, 2017). Further, compost additions have been suggested as a potential C sequestration practice (Calleja-Cervantes et al., 2015a; Longbottom and Petrie, 2015), although N_2O (denitrification) and CO_2 (respiration) emissions may be increased in compost treated plots as compared

to controls or grower standard NPK practices (Calleja-Cervantes et al., 2015a). Thus, many of the improvements to soil health and the manifestation of terroir from compost additions to be discussed here are rooted in the addition of C and increases to SOC/SOM from compost addition.

Addition of composts routinely increases soil microbial biomass carbon (MBC) and respiration as measured by CO_2 evolution (Bustamante et al., 2011; Rubio et al., 2013; Wilson et al., 2016; Gaiotti et al., 2017). An increase in the size and activity of the microbial biomass can have beneficial effects to nutrient cycling, especially in degraded soils (Ros, 2003; Lazcano et al., 2013). Compost application can also change the structure of the soil microbial community; for instance, in a calcareous vineyard sheep compost enhanced soil microbial activity and shifted bacterial composition from oligotrophic to copiotrophic, as shown by differences in 16S rRNA gene sequences (Calleja-Cervantes et al., 2015a). A robust and diverse microbial community is a hallmark of healthy soils. However, changes in the soil microbial community resulting from application of composts can be different for different soil types. For example, organic inputs increased MBC in fine textured non-calcareous soils, but not in calcareous soils in France (Salomé et al., 2016). Increases in MBC are tied to the rate of C applied, but less to the source of C. Inputs from diverse composts (mushroom compost, farmyard manure, and vine pruning waste compost) had a similar response in MBC. Increases in the rate of C applied from composts applied lead to incremental increases in MBC (Morlat and Chaussod, 2008; Peregrina et al., 2012). Both single doses, and long-term applications increases MBC (Morlat and Chaussod, 2008; Rubio et al., 2013). Other investigations have reported no response or a modest non-significant responses of compost addition to MBC (Nendel and Reuter, 2007; Gaiotti et al., 2017). Differences in the magnitude of the MBC response from compost addition is tied to the antecedent soil health, particularly the physical and biological conditions, as well as the source and rate of compost additions (Gaiotti et al., 2017). More research is needed to connect compost addition rates, soil types and soil health objectives and potential benefits from increased MBC.

Compost application routinely increases soil N, and this increase is incremental with compost application rate (Korboulewsky et al., 2002; Peregrina et al., 2012). Peregrina et al. (2012) reported increases in N and SOC with increases in the rate of fresh and composted mushroom substrate applied. Application of various sources of organic materials, including composted sewage sludges, mushroom composts, composted cattle manure, winery wastes and pruning wastes routinely increase soil total N (Larchevêque et al., 2006; Morlat and Chaussod, 2008; Calleja-Cervantes et al., 2015a; Gaiotti et al., 2017). Mugnai et al. (2012) reported an increase in total N over a positive control (NPK) following 9 years of compost grape waste application, with NH_4–N favored over NO_3–N in compost treated plots, with the opposite true in the mineral nutrient positive control (grower standard NPK practice). Long term and continuous applications of cattle manure in excess of crop demand, may result in N build up, N leaching and potential suppressive effects on yield and quality (Morlat and Chaussod, 2008; Morlat and Symoneaux, 2008). While application of composts and other organic materials generally increases soil total N, differences exist in the amounts of organic N versus inorganic N, as well as the timing of release soluble N from mineralization of added organic inputs.

Initial compost applications result in a quick pulse of inorganic N followed by a prolonged period of elevated organic N. Synchronizing mineralization of compost N and the timing of high N demand in winegrapes is an area of ongoing investigation. In a 3-year study applying a range of compost types to a calcareous soil, Bustamante et al. (2011) reported that diverse sources of compost result in an initial spike in soluble N that is tempered with time, while organic N tends to remain elevated. They concluded that excess N was not observed in the soil, suggesting that the pulse of inorganic N following mineralization was utilized by the vines. Similar results were reported by Rubio et al. (2013), after application of a variety of compost sources including pruning wastes, winery wastes (pumice), sheep manure, cattle manure and mixes of manures and pumice. Similarly, application of vermicompost and vine shoot compost led to an initial large increases in extractable N, with subsequent applications leading to progressively smaller increases in extractable N, but higher values of microbial biomass N (Mondini et al., 2018). Potentially, initial applications of compost lead to initial increases in inorganic N before the additional C increased the size of the microbial biomass, and additional N resources are either assimilated into the microbial biomass, taken up by vines or lost through denitrification (Korboulewsky et al., 2002) applied three rates of sewage sludge to a French vineyard and reported an initial spike in inorganic N (NO_3 and NH_4), which subsided with increased application. Broadly, amounts of N remain elevated in compost treated plots compared to controls, and inorganic and organic N increased incrementally with compost application rate.

In winegrapes N uptake has been observed in two major periods, one from budbreak to veraison, and the second post-harvest, with as much as 34% of the total seasonal N uptake in this period (Conradie, 2017). N pulses following compost application are likely to be utilized by the vine, especially if N deficiencies exits. Winegrapes benefit from an extensive established root system such that the soluble N pulse following application can be utilized by vines at budbreak into flowering, whereas spring N pulses may be lost in shallow rooted row crop systems. This is particularly true if a restorative effect is desired from application of compost to established vines, where roots are eager for fresh nutrient inputs. For example, in an investigation of compost application rates to a degraded vineyard soil with established, underperforming vines, compost application in the dormant season resulted in a significant increase petiole N in the following growing season, with this increase incremental with application rate (significant dose response). Application of composts to established vines will result in uptake of the initial pulse of soluble N. Conversely large applications of high N composts (composted manures) at preplant may result in losses of N to the environment.

Soil phosphorus (P) is typically increased as the result of compost application (Korboulewsky et al., 2002; Morlat and Chaussod, 2008; Bustamante et al., 2011; Wilson et al., 2016),

due to many factors including the additional P applied in the compost, the chelation of active Al or Ca by organic acids and other decomposition products, and competitive inhibition of P-sorption sites by organic acid anions (Hue, 1992; Delgado et al., 2002). Winegrapes have relatively low P demands (Schreiner and Osborne, 2018). The combined effect of relatively low P demand, and increased P availability due to P sorption inhibition, can lead to excess P following compost application (Korboulewsky et al., 2002; Wilson et al., 2016). Care should be taken, especially when composts or other organic materials are applied to meet N demands, that excess P is not applied (Korboulewsky et al., 2002). Nonetheless, P deficiency in winegrapes has been reported in certain soil types with less than 10 mg P per kg soil (Olsen-P) (Skinner et al., 1988). P deficiency can lead to reduced fruit set, yields, and vegetative growth (Skinner et al., 1988). Yield reduction from P deficiency is likely due to deleterious effects on the initiation and differentiation of bud primordia. P deficiency is manifest in blotchy red interveins, which can be mistaken for leafroll disease or red blotch disease. Soil P dynamics are directly tied to the soil component of terroir, with certain soil types, particularly red, clay rich soils from high Fe/Al parent materials (basalts and andesites), and volcanic ash soils, exhibiting P deficiency and higher P sorption capacities. In these instances, compost application can be a favorable management strategy to relieve P deficiency, due to the combined beneficial effect of applied SOM and applied P. Management of P deficiency in winegrapes is largely driven by the terroir of the soil, as influenced by pedogensis. For example, weathered soils derived from low P lithologies, such as granite, may be P deficient, but also have low P fixation capacities, and hence would respond well to P applications (Wilson et al., 2016, 2017). Conversely, P deficient soils with high P sorption capacities, such as volcanic ash soils, will require higher applications of compost to overcome the P fixation capacity. In most instances applying composts to meet P demand will result in lower P sorption capacities, and higher P availability, than mineral fertilizers alone due to competitive inhibition of anion sorption sites (Hue, 1992; Hunt et al., 2007; Wilson et al., 2016). The management of P, both with and without composts, is dependent on the terroir of the site, as manifested by soil genesis, particularly soil mineralogy and acidity/alkalinity.

In winegrapes, reduced K supply can lead to premature leaf drop, and negative effects on yield and vegetative growth (Christensen and Peacock, 2000). Potassium is the major cation in grape juice and musts and has a significant effect on juice pH (Mpelasoka et al., 2003). Excess K has been connected to undesirably high wine pH, reduced wine stability and declines in color quality (Mpelasoka et al., 2003). Excessive K in berries may lead to lower levels of the more desirable tartaric acid, altering the perception of flavor (Mpelasoka et al., 2003). In a wide range of studies with many different sources, compost application usually increases soil extractable K. Many different types of composts, cattle manures, winery wastes, sheep manures, municipal solid wastes, garden green waste mulches, mushroom composts, and mixes of these materials result in elevated K status in soils (Pinamonti, 1998; Larchevêque et al., 2006; Morlat and Chaussod, 2008; Bustamante et al., 2011; Chan and Fahey, 2011; Calleja-Cervantes et al., 2015a,b). This is particularly true for composts with a significant proportion of winery wastes which are higher in soluble K, and composts derived from manures (Bustamante et al., 2008; Rubio et al., 2013). Compost application leads to incremental increases in soil K, with very high levels of soil K corresponding to higher application rates (Morlat and Chaussod, 2008; Chan and Fahey, 2011).

When available, vines tend to uptake excess K, such that when soil K was increased from compost addition, plant tissue K also increases (Pinamonti, 1998; Morlat and Chaussod, 2008; Chan and Fahey, 2011). Given that berries are strong sinks for K, excess K supplied to vines results in larger amounts of K in berries (Pinamonti, 1998; Mpelasoka et al., 2003; Morlat and Symoneaux, 2008; Chan and Fahey, 2011). The strongest effect of compost applications to vineyard soils on wine quality may be from K supply, with increased K observed in grapes and musts as the result of compost addition (Pinamonti, 1998; Morlat and Symoneaux, 2008). With respect to terroir, Chan and Fahey (2011) noted that the treatment effect of composts on berry K was much less than the site effect on berry K. This suggest that, especially with respect to K, the inherent terroir, related to soil mineralogy and parent material, may be a greater factor in soil, vine and berry K status than the K applied in composts. However, further research is required to understand the variability in soil and vine K status attributable to variability in site characteristics and terroir. While several studies report increased berry K from increased extractable soil K following compost addition (Rubio et al., 2013) observed significant increases in soil K, but not in berry K. Conversely, lower rates of vermicompost and compost additions did not result in increased soil K status nor vine K status (Martinez et al., 2018). Mpelasoka et al. (2003) notes that the relationship between K supply in soils and K in berries and tissues is not always absolute. Differences in application rate, source, timing, irrigation, as well as vine parameters such as root architecture and the initial plant nutrient status will influence the uptake and availability of K. Given the significant variability in K status of soils due to differences in their terroir and pedogenic environment (mineralogy and parent material) as well as the effect of composts on soil K status, significant knowledge gaps exits connecting antecedent soil K, K supplied in composts, and the connection between soil K, berry K, must K, and wine quality.

In vineyard soils, increases in organic C from compost addition improve many soil physical properties. For example, in Mediterranean-type soils, compost additions increase aggregate stability (Celik et al., 2004; Kong et al., 2005; Goulet et al., 2006). The organic C fraction of soils is lighter than the mineral fraction and compost additions to vineyard soils typically reduce the soil bulk density (Celik et al., 2004; Morlat and Chaussod, 2008; Brown and Cotton, 2011; Salomé et al., 2016; Ramos, 2017). Both reductions in bulk density and improved aggregation lead to improved soil porosity, with improved porosity reported following compost addition (Pérès et al., 1998; Pinamonti, 1998). Soil temperature fluctuations are also reduced from application of compost mulches, especially compared to plastic row covers (Pinamonti, 1998). Improvements to soil structure and aggregation as well as improvements to porosity, lead to improvements in soil water holding capacity. Improved water holding capacity following compost addition is widely

reported (Pinamonti, 1998; Morlat and Chaussod, 2008; Brown and Cotton, 2011; Ramos, 2017; Mondini et al., 2018). Of all soil properties, soil water has the most significant effect on vine growth, vigor, berry formation and subsequent effects on wine quality (Keller, 2005, 2010). Excess water, which leads to excess shoot growth, has been linked to declines in wine quality (Hepner et al., 1985; Wheeler and Pickering, 2003). However, concerns over climate change, drought and water use efficiency suggest that improvements to soil water holding capacity following compost addition are likely to be beneficial dependent on soil type and edaphic conditions. For example, Ramos (2017) reported significant improvements to soil water holding capacity and infiltration rate following compost addition to degraded vineyard soils, with the positive effects of compost addition on per vine yield stronger in drier years. These data suggest that, in degraded soils low in organic matter, increasing SOC from composts addition may provide some resilience to drought conditions. Similarly, in degraded vineyard soils application of vine shoot composts, but not vermicompost, lead to improved soil water holding capacity (Mondini et al., 2018). Dependent on the rate and amount of compost, compost application can be expected to improve soil water holding capacity, especially with repeated applications of high C sources. Increases in soil water holding capacity following compost addition can have significant effects on vine performance. Care should be taken by viticulturalists to mediate irrigation practices following sustained application of composts. Nonetheless, compost addition to vineyard soils is promising to mitigate soil drought and improve water use efficiency. Changes and improvements to vine water use efficiency following application of diverse sources of compost requires further investigation.

Improvements to vineyard soil health are generally consistent following compost application, improving soil biological, chemical, and physical characteristics. However, there is a view among viticulturalists that compost applications may have a negative effect on quality due to excess vigor and an imbalance between shoot growth and yield, and that the clear benefits to vineyard soil health from compost application may be outweighed by the negative effects on vine balance and grape quality. Yet, this is not borne out of the available data. For example, several studies have shown no significant changes to vine balance or juice quality following compost application (Pinamonti, 1998; Bartoli and Dousset, 2011; Mugnai et al., 2012; Mondini et al., 2018). Mugnai et al. (2012) applied municipal green waste compost to a chardonnay vineyard in the Tuscany region of Italy and conclude that, using measures of both leaf area and leaf chlorophyll, excess vigor, yields, and grape quality were not significantly affected by compost addition. Similarly, Pinamonti (1998) report no negative effects to vine balance (yields/pruning weights), total soluble solids (TSS) or pH following compost addition. Where improved water status is implicated in yield or shoot growth increases in some studies (Pinamonti, 1998; Ramos, 2017), soil N status drives increased yields and shoot growth in others. (Gaiotti et al., 2017) applied composted cattle manure and composted vine pruning wastes over a 5-year period and reported significant increases in vine growth and yield, without significant changes to vine balance (per vine yield/pruning weights). They note increased vigor in treatments containing composted cattle manure, a result they ascribe to faster mineralization rates, and more available N. In a similar, but single season study Rubio et al. (2013) applied diverse composts (citrus waste, winery waste, composted cattle manure, vine pruning wastes, and combinations of materials) and reported increases to yields, especially for mixes containing composted cattle manure, a response they also attribute to the elevated soil N status. With respect to juice chemistry, TSS was unaffected and the total polyphenol index was influenced by compost treatment, but trends were unclear. While increased N status led to increased yield in some studies, excess N was implicated in yield decline in others (Morlat, 2008). There, sustained applications of high rates (20 t ha^{-1} for 28 years) of relatively high N composted manures had a negative effect on yields and pruning weights, potentially due to N toxicity. Conversely, lower application rates of vine pruning compost (with higher C:N ratios) had a positive effect on yields and pruning weights. Nonetheless, no changes were reported to vine balance after 28 years of continued compost application from any source trialed. With respect to juice chemistry, Morlat (2008) report that total anthocyanins and TSS were decreased, while pH and K^+ were increased in berries following addition of high rates of composted manures. In contrast, in a 3-year investigation of irrigation strategies with and without compost addition, (Cirigliano et al., 2017) found improved anthocyanin and other polyphenol contents in berries following compost addition compared to treatments without compost. Their data suggest that in drip irrigated Mediterranean-type climates, compost application can improve grape quality over irrigation alone, perhaps due to better infiltration and water holding capacity in compost treated vines. Contrasting results on the impact of composts on vine growth and yield, as well as on grape quality, especially polyphenol compositions, requires further investigation.

Compost applications can lead to improved yields and pruning weights, without detrimental effects to grape quality (pH, TSS, and TA), although effects to anthocyanins and other polyphenols are unclear. Increases in yields and pruning weights are tied to increases in soil water status and soil N status. Evaluating the existing soil conditions, as well as available water for irrigation and yield and quality objectives, ultimately informs the compost approach adopted. For example, in established normally performing vineyards, success has been accomplished with lower N inputs, such as vine pruning waste composts (Morlat, 2008; Morlat and Chaussod, 2008; Gaiotti et al., 2017). If a significant yield response is desired, or if soils are degraded, composts richer in plant available nutrients can be applied with success (Ramos, 2017; Mondini et al., 2018). The beneficial effect of compost application on yields in drought years noted by Mondini et al. (2018) and Ramos (2017) highlights a potentially significant benefit from compost addition in the face of water shortages and requires further investigation. Broadly, more research is needed to quantify yield, pruning weight, and grape quality responses from application of diverse composts in a variety of soil types, to solidify our understanding of the effect of composts in viticulture.

CONCLUSION

As we increase our understanding of the fundamental role of soils and soil health in long term climate regulation, crop yields and quality, there is an urgent need to understand how the dynamic and inherent aspects of soil health may overlap with the concept of terroir in wine grape production. In this review we show that, because of their impact on vine health and grape quality, the physical, chemical, and biological aspects of soil health overlap clearly with the soil related aspects of the *terroir*. Furthermore, we find potential for the expansion of the terroir concept by incorporating dynamic aspects of soil health such as SOM, soil C and soil biota which influence vine performance, potentially affecting wine quality. In spite of this, there are no crop specific guidelines or reference values that would help growers manage soil health for an optimum expression of the terroir. These guidelines need to be established by defining the meaning and main functions of a healthy soil in grapevines as compared to other crops and incorporating regional variability and site-specific needs.

Conservation of soil health needs to be prioritized rather than restoration of degraded soils. The use of cover crops and compost, supports the physical, chemical, and biological aspects of soil health, therefore contributing to the expression of the soil related aspects of the terroir. However, there are outstanding knowledge gaps that need to be addressed in order to implement grapevine specific best management practices. In particular, more information is needed on different cover crop species and mixes, their effects on vine nutrient and water uptake, the suitability of to boost different aspects of a healthy soil, and the consequences for grape and wine quality. In regard to the use of cover crops, we need a better understanding of the effects of different termination strategies (mowing, tilling, and grazing) and timing on soil health, crop yield and quality. Compost application clearly improves soil health, yet there is large variability in the observed effects potentially associated with the use of different feedstocks, placement, application rate and timing. The role of these factors needs to be understood in order to manage soil health for the maximum expression of the terroir.

AUTHOR CONTRIBUTIONS

CL and CD developed the idea for this review manuscript. CL, SW, and CD performed the literature review, and discussed and collected the data.

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REFERENCES

Aljibury, F. K., and Christensen, L. P. (1972). Water penetration of vineyard soils as modified by cultural practices. *Am. J. Enol. Vitic.* 23, 35–39.

Bardgett, R. D., and van der Putten, W. H. (2014). Belowground biodiversity and ecosystem functioning. *Nature* 515, 505–511. doi: 10.1038/nature13855

Bartoli, F., and Dousset, S. (2011). Impact of organic inputs on wettability characteristics and structural stability in silty vineyard topsoil. *Eur. J. Soil Sci.* 62, 183–194. doi: 10.1111/j.1365-2389.2010.01337.x

Battany, M. C., and Grismer, M. E. (2000). Rainfall runoff and erosion in Napa Valley vineyards: effects of slope, cover and surface roughness. *Hydrol. Process.* 14, 1289–1304. doi: 10.1002/(sici)1099-1085(200005)14:7<1289::aid-hyp43>3.0.co;2-r

Belda, I., Zarraonaindia, I., Perisin, M., Palacios, A., and Acedo, A. (2017). From vineyard soil to wine fermentation: microbiome approximations to explain the "terroir" Concept. *Front. Microbiol.* 8:821. doi: 10.3389/fmicb.2017.00821

Bell, S.-J., and Henschke, P. A. (2005). Implications of nitrogen nutrition for grapes, fermentation and wine. *Aust. J. Grape Wine Res.* 11, 242–295. doi: 10.1111/j.1755-0238.2005.tb00028.x

Berendsen, R. L., Pieterse, C. M. J., and Bakker, P. A. H. M. (2012). The rhizosphere microbiome and plant health. *Trends Plant Sci.* 17, 478–486. doi: 10.1016/j.tplants.2012.04.001

Biddoccu, M., Ferraris, S., Pitacco, A., and Cavallo, E. (2017). Temporal variability of soil management effects on soil hydrological properties, runoff and erosion at the field scale in a hillslope vineyard, North-West Italy. *Soil Tillage Res.* 165, 46–58. doi: 10.1016/j.still.2016.07.017

Blankinship, J. C., Fonte, S. J., Six, J., and Schimel, J. P. (2016). Plant versus microbial controls on soil aggregate stability in a seasonally dry ecosystem. *Geoderma* 272, 39–50. doi: 10.1016/j.geoderma.2016.03.008

Blavet, D., De Noni, G., Le Bissonnais, Y., Leonard, M., Maillo, L., Laurent, J. Y., et al. (2009). Effect of land use and management on the early stages of soil water erosion in French Mediterranean vineyards. *Soil Tillage Res.* 106, 124–136. doi: 10.1016/j.still.2009.04.010

Bokulich, N. A., Collins, T. S., Masarweh, C., Allen, G., Heymann, H., Ebeler, S. E., et al. (2016). Associations among wine grape microbiome, metabolome, and fermentation behavior suggest microbial contribution to regional wine characteristics. *mBio* 7:e00631-16. doi: 10.1128/mBio.00631-16

Bokulich, N. A., Thorngate, J. H., Richardson, P. M., and Mills, D. A. (2013). Microbial biogeography of wine grapes is conditioned by cultivar, vintage, and climate. *Proc. Natl. Acad. Sci. U.S.A.* 111, 139–148. doi: 10.1073/pnas.1317377110

Booth, M. S., Stark, J. M., and Rastetter, E. (2005). Controls on nitrogen cycling in terrestrial ecosystems: a synthetic analysis of literature data. *Ecol. Monogr.* 75, 139–157. doi: 10.1890/04-0988

Borrelli, P., Robinson, D. A., Fleischer, L. R., Lugato, E., Ballabio, C., Alewell, C., et al. (2017). An assessment of the global impact of 21st century land use change on soil erosion. *Nat. Commun.* 8:2013. doi: 10.1038/s41467-017-02142-7

Bowles, T. M., Hollander, A. D., Steenwerth, K., and Jackson, L. E. (2015). Tightly-coupled plant-soil nitrogen cycling: comparison of organic farms across an agricultural landscape. *PLoS One* 10:e0131888. doi: 10.1371/journal.pone.0131888

Bowles, T. M., Jackson, L. E., Loeher, M., and Cavagnaro, T. R. (2017). Ecological intensification and arbuscular mycorrhizas: a meta-analysis of tillage and cover crop effects. *J. Appl. Ecol.* 54, 1785–1793. doi: 10.1111/1365-2664.12815

Brady, N. C., and Weil, R. R. (2016). *The Nature and Properties of Soils*, 15th Edn. Columbus, OH: Pearson.

Bramley, R. G. V., and Janik, L. J. (2005). Precision agriculture demands a new approach to soil and plant sampling and analysis—examples from Australia. *Commun. Soil Sci. Plant Anal.* 36, 9–22. doi: 10.1081/CSS-200042958

Brevik, E. C., Steffan, J. J., Rodrigo-Comino, J., Neubert, D., Burgess, L. C., and Cerdà, A. (2019). Connecting the public with soil to improve human health: connect public with soil to improve human health. *Eur. J. Soil Sci.* 70, 898–910. doi: 10.1111/ejss.12764

Brown, S., and Cotton, M. (2011). Changes in soil properties and carbon content following compost application: results of on-farm sampling. *Compost Sci. Util.* 19, 87–96. doi: 10.1080/1065657X.2011.10736983

Brunori, E., Farina, R., and Biasi, R. (2016). Sustainable viticulture: the carbon-sink function of the vineyard agro-ecosystem. *Agric. Ecosyst. Environ.* 223, 10–21. doi: 10.1016/j.agee.2016.02.012

Bünemann, E. K., Bongiorno, G., Bai, Z., Creamer, R. E., De Deyn, G., de Goede, R., et al. (2018). Soil quality – A critical review. *Soil Biol. Biochem.* 120, 105–125. doi: 10.1016/j.soilbio.2018.01.030

Burns, K. N., Bokulich, N. A., Cantu, D., Greenhut, R. F., Kluepfel, D. A., O'Geen, A. T., et al. (2016). Vineyard soil bacterial diversity and composition revealed by 16S rRNA genes: differentiation by vineyard management. *Soil Biol. Biochem.* 103, 337–348. doi: 10.1016/j.soilbio.2016.09.007

Bustamante, M. A., Moral, R., Paredes, C., Pérez-Espinosa, A., Moreno-Caselles, J., and Pérez-Murcia, M. D. (2008). Agrochemical characterisation of the solid by-products and residues from the winery and distillery industry. *Waste Manag.* 28, 372–380. doi: 10.1016/j.wasman.2007.01.013

Bustamante, M. A., Said-Pullicino, D., Agulló, E., Andreu, J., Paredes, C., and Moral, R. (2011). Application of winery and distillery waste composts to a Jumilla (SE Spain) vineyard: effects on the characteristics of a calcareous sandy-loam soil. *Agric. Ecosyst. Environ.* 140, 80–87. doi: 10.1016/j.agee.2010.11.014

Butterbach-Bahl, K., Baggs, E. M., Dannenmann, M., Kiese, R., and Zechmeister-Boltenstern, S. (2013). Nitrous oxide emissions from soils: how well do we understand the processes and their controls? *Philos. Trans. R. Soc. B Biol. Sci.* 368:20130122. doi: 10.1098/rstb.2013.0122

Calleja-Cervantes, M. E., Fernández-González, A. J., Irigoyen, I., Fernández-López, M., Aparicio-Tejo, P. M., and Menéndez, S. (2015a). Thirteen years of continued application of composted organic wastes in a vineyard modify soil quality characteristics. *Soil Biol. Biochem.* 90, 241–254. doi: 10.1016/j.soilbio.2015.07.002

Calleja-Cervantes, M. E., Menéndez, S., Fernández-González, A. J., Irigoyen, I., Cibriáin-Sabalza, J. F., Toro, N., et al. (2015b). Changes in soil nutrient content and bacterial community after 12 years of organic amendment application to a vineyard: compost changes soil's mineral content and microbial community. *Eur. J. Soil Sci.* 66, 802–812. doi: 10.1111/ejss.12261

Celette, F., and Gary, C. (2013). Dynamics of water and nitrogen stress along the grapevine cycle as affected by cover cropping. *Eur. J. Agron.* 45, 142–152. doi: 10.1016/j.eja.2012.10.001

Celette, F., Gaudin, R., and Gary, C. (2008). Spatial and temporal changes to the water regime of a Mediterranean vineyard due to the adoption of cover cropping. *Eur. J. Agron.* 29, 153–162. doi: 10.1016/j.eja.2008.04.007

Celik, I., Ortas, I., and Kilic, S. (2004). Effects of compost, mycorrhiza, manure and fertilizer on some physical properties of a Chromoxerert soil. *Soil Tillage Res.* 78, 59–67. doi: 10.1016/j.still.2004.02.012

Chan, K. Y., and Fahey, D. J. (2011). Effect of composted mulch application on soil and wine grape potassium status. *Soil Res.* 49, 455–461. doi: 10.1071/SR11084

Chou, M.-Y., Vanden Heuvel, J., Bell, T. H., Panke-Buisse, K., and Kao-Kniffin, J. (2018). Vineyard under-vine floor management alters soil microbial composition, while the fruit microbiome shows no corresponding shifts. *Sci. Rep.* 8:11039. doi: 10.1038/s41598-018-29346-1

Christensen, L. P., and Peacock, W. L. (2000). "Mineral nutrition and fertilization," in *Raisin Production Manual*, ed. L. P. Christensen (Oakland, CA: University of California Agriculture and Natural Resources), 102–114.

Cirigliano, P., Vincenza Chiriacò, M., Nunez, A., Dal Monte, G., and Labagnara, T. (2017). Efecto combinado de la aplicación de riego y compost sobre la composición de la baya Montepulciano en un entorno volcánico de la región de Lacio (Italia central). *Cienc. E Investig. Agrar.* 44, 195–206. doi: 10.7764/rcia.v44i2.1691

Conradie, W. J. (2017). Seasonal uptake of nutrients by Chenin Blanc in sand culture: II. Phosphorus, potassium, calcium and magnesium. *S. Afr. J. Enol. Vitic.* 2, 7–13. doi: 10.21548/2-1-2403

Costantini, E. A. C., and Bucelli, P. (2014). "Soil and Terroir," in *Soil Security for Ecosystem Management*, eds S. Kapur and S. Erşahin (Cham: Springer), 97–133. doi: 10.1007/978-3-319-00699-4_6

Delgado, A., Madrid, A., Kassem, S., Andreu, L., and del Campillo, M. D. C. (2002). Phosphorus fertilizer recovery from calcareous soils amended with humic and fulvic acids. *Plant Soil* 245, 277–286. doi: 10.1023/A:1020445710584

Delpuech, X., and Metay, A. (2018). Adapting cover crop soil coverage to soil depth to limit competition for water in a Mediterranean vineyard. *Eur. J. Agron.* 97, 60–69. doi: 10.1016/j.eja.2018.04.013

Doran, J. W. (1994). Defining and assessing soil quality. *SSSA Spec. Publ.* 35, 3–21.

Drinkwater, L. E., and Snapp, S. S. (2007). Nutrients in agroecosystems: rethinking the management paradigm. *Adv. Agron.* 92, 163–186. doi: 10.1016/S0065-2113(04)92003-2

Dunn, M., Ulrich-Schad, J. D., Prokopy, L. S., Myers, R. L., Watts, C. R., and Scanlon, K. (2016). Perceptions and use of cover crops among early adopters: findings from a national survey. *J. Soil Water Conserv.* 71, 29–40. doi: 10.2489/jswc.71.1.29

FAO, and ITPS (2015). *Status of the World's Soil Resources: Main Report*. Rome: FAO.

Fierer, N. (2017). Embracing the unknown: disentangling the complexities of the soil microbiome. *Nat. Rev. Microbiol.* 15, 579–590. doi: 10.1038/nrmicro.2017.87

Fine, A. K., van Es, H. M., and Schindelbeck, R. R. (2017). Statistics, scoring functions, and regional analysis of a comprehensive soil health database. *Soil Sci. Soc. Am. J.* 81, 589–601. doi: 10.2136/sssaj2016.09.0286

Forte, V., Angelini, E., Maixner, M., and Borgo, M. (2010). Preliminary results on population dynamics and host plants of Hyalesthes obsoletus in North-Eastern Italy. *Vitis* 49, 39–42.

Gaiotti, F., Marcuzzo, P., Belfiore, N., Lovat, L., Fornasier, F., and Tomasi, D. (2017). Influence of compost addition on soil properties, root growth and vine performances of *Vitis vinifera* cv Cabernet sauvignon. *Sci. Hortic.* 225, 88–95. doi: 10.1016/j.scienta.2017.06.052

Galati, A., Crescimanno, M., Gristina, L., Keesstra, S., and Novara, A. (2016). Actual provision as an alternative criterion to improve the efficiency of payments for ecosystem services for C sequestration in semiarid vineyards. *Agric. Syst.* 144, 58–64. doi: 10.1016/j.agsy.2016.02.004

García-Díaz, A., Bienes, R., Sastre, B., Novara, A., Gristina, L., and Cerdà, A. (2017). Nitrogen losses in vineyards under different types of soil groundcover. A field runoff simulator approach in central Spain. *Agric. Ecosyst. Environ.* 236, 256–267. doi: 10.1016/j.agee.2016.12.013

Garland, G. M., Suddick, E., Burger, M., Horwath, W. R., and Six, J. (2011). Direct N2O emissions following transition from conventional till to no-till in a cover cropped Mediterranean vineyard (*Vitis vinifera*). *Agric. Ecosyst. Environ.* 144, 423–428. doi: 10.1016/j.agee.2011.11.001

Gilbert, J. A., van der Lelie, D., and Zarraonaindia, I. (2014). Microbial terroir for wine grapes. *Proc. Natl. Acad. Sci. U.S.A.* 111, 5–6. doi: 10.1073/pnas.1320471110

Goulet, E., Dousset, S., Chaussod, R., Bartoli, F., Doledec, A. F., and Andreux, F. (2006). Water-stable aggregates and organic matter pools in a calcareous vineyard soil under four soil-surface management systems. *Soil Use Manag.* 20, 318–324. doi: 10.1111/j.1475-2743.2004.tb00376.x

Gulick, S. H., Grimes, D. W., Goldhamer, D. A., and Munk, D. S. (1994). Cover-crop-enhanced water infiltration of a slowly permeable fine sandy loam. *Soil Sci. Soc. Am. J.* 58, 1539–1546. doi: 10.2136/sssaj1994.03615995005800050038x

Guzmán, G., Cabezas, J. M., Sánchez-Cuesta, R., Lora, Á., Bauer, T., Strauss, P., et al. (2019). A field evaluation of the impact of temporary cover crops on soil properties and vegetation communities in southern Spain vineyards. *Agric. Ecosyst. Environ.* 272, 135–145. doi: 10.1016/j.agee.2018.11.010

Haygarth, P. M., and Ritz, K. (2009). The future of soils and land use in the UK: soil systems for the provision of land-based ecosystem services. *Land Use Policy* 26, S187–S197. doi: 10.1016/j.landusepol.2009.09.016

Hepner, Y., Bravdo, B., Loinger, C., Cohen, S., and Tabacman, H. (1985). Effect of drip irrigation schedules on growth, yield, must composition and wine quality of cabernet sauvignon. *Am. J. Enol. Vitic.* 36, 77–85.

Hue, N. V. (1992). Correcting soil acidity of a highly weathered Ultisol with chicken manure and sewage sludge. *Commun. Soil Sci. Plant Anal.* 23, 241–264. doi: 10.1080/00103629209368586

Hunt, J. F., Ohno, T., He, Z., Honeycutt, C. W., and Dail, D. B. (2007). Inhibition of phosphorus sorption to goethite, gibbsite, and kaolin by fresh and decomposed organic matter. *Biol. Fertil. Soils* 44, 277–288. doi: 10.1007/s00374-007-0202-1

Karlen, D. L., Veum, K. S., Sudduth, K. A., Obrycki, J. F., and Nunes, M. R. (2019). Soil health assessment: past accomplishments, current activities, and future opportunities. *Soil Tillage Res.* 195:104365. doi: 10.1016/j.still.2019.104365

Karlen, D., and Rice, C. (2015). Soil degradation: Will humankind ever learn? *Sustainability* 7, 12490–12501. doi: 10.3390/su70912490

Keesstra, S. D., Bouma, J., Wallinga, J., Tittonell, P., Smith, P., Cerdà, A., et al. (2016). The significance of soils and soil science towards realization of the

United Nations Sustainable Development Goals. *Soil* 2, 111–128. doi: 10.5194/soil-2-111-2016

Keesstra, S., Mol, G., de Leeuw, J., Okx, J., Molenaar, C., de Cleen, M., et al. (2018). Soil-related sustainable development goals: four concepts to make land degradation neutrality and restoration work. *Land* 7, 1–20. doi: 10.3390/land7040133

Keller, M. (2005). Deficit irrigation and vine mineral nutrition. *Am. J. Enol. Vitic.* 56, 267–283.

Keller, M. (2010). *The Science of Grapevines: Anatomy and Physiology*. Burlington, MA: Academic Press.

Kern, J. S. (1995). Evaluation of soil water retention models based on basic soil physical properties. *Soil Sci. Soc. Am. J.* 59, 1134–1141. doi: 10.2136/sssaj1995.03615995005900040027x

Kögel-Knabner, I. (2017). The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter: fourteen years on. *Soil Biol. Biochem.* 105, A3–A8. doi: 10.1016/j.soilbio.2016.08.011

Kong, A. Y. Y., Six, J., Bryant, D. C., Denison, R. F., and van Kessel, C. (2005). The relationship between carbon input, aggregation, and soil organic carbon stabilization in sustainable cropping systems. *Soil Sci. Soc. Am. J.* 69, 1078–1085. doi: 10.2136/sssaj2004.0215

Korboulewsky, N., Dupouyet, S., and Bonin, G. (2002). Environmental risks of applying sewage sludge compost to vineyards: carbon, heavy metals, nitrogen, and phosphorus accumulation. *J. Environ. Qual.* 31, 1522–1527. doi: 10.2134/jeq2002.1522

Kroodsma, D. A., and Field, C. B. (2006). Carbon sequestration in california agriculture, 1980–2000. *Ecol. Appl.* 16, 1975–1985. doi: 10.1890/1051-0761(2006)016[1975:csica]2.0.co;2

Ladha, J. K., Pathak, H. J., Krupnik, T., Six, J., and van Kessel, C. (2005). Efficiency of fertilizer nitrogen in cereal production: retrospects and prospects. *Adv. Agron.* 87, 85–156. doi: 10.1016/S0065-2113(05)87003-8

Lal, R. (2004). Soil carbon sequestration to mitigate climate change. *Geoderma* 123, 1–22. doi: 10.1016/j.geoderma.2004.01.032

Larchevêque, M., Baldy, V., Montès, N., Fernandez, C., Bonin, G., and Ballini, C. (2006). Short-term effects of sewage-sludge compost on a degraded mediterranean soil. *Soil Sci. Soc. Am. J.* 70, 1178–1188. doi: 10.2136/sssaj2005.0115

Lazcano, C., Gómez-Brandón, M., Revilla, P., and Domínguez, J. (2013). Short-term effects of organic and inorganic fertilizers on soil microbial community structure and function: a field study with sweet corn. *Biol. Fertil. Soils* 49, 723–733. doi: 10.1007/s00374-012-0761-7

Lehmann, J., and Kleber, M. (2015). The contentious nature of soil organic matter. *Nature* 528, 60–68. doi: 10.1038/nature16069

Longbottom, M. L., and Petrie, P. R. (2015). Role of vineyard practices in generating and mitigating greenhouse gas emissions: greenhouse gas emissions in vineyards. *Aust. J. Grape Wine Res.* 21, 522–536. doi: 10.1111/ajgw.12197

López-Vicente, M., and Álvarez, S. (2018). Stability and patterns of topsoil water content in rainfed vineyards, olive groves, and cereal fields under different soil and tillage conditions. *Agric. Water Manag.* 201, 167–176. doi: 10.1016/j.agwat.2018.02.004

Mackenzie, D. E., and Christy, A. G. (2005). The role of soil chemistry in wine grape quality and sustainable soil management in vineyards. *Water Sci. Technol.* 51, 27–37. doi: 10.2166/wst.2005.0004

Maltman, A. (2013). Minerality in wine: a geological perspective. *J. Wine Res.* 24, 169–181. doi: 10.1080/09571264.2013.793176

Martinez, L. E., Vallone, R. C., Piccoli, P. N., and Ratto, S. E. (2018). Assessment of soil properties, plant yield and composition, after different type and applications mode of organic amendment in a vineyard of Mendoza, Argentina. *Rev. Fac. Cienc. Agrar.* 50, 17–32.

Martínez-Casasnovas, J. A., and Ramos, M. C. (2006). The cost of soil erosion in vineyard fields in the Penedès–Anoia Region (NE Spain). *Catena* 68, 194–199. doi: 10.1016/j.catena.2006.04.007

Medrano, H., Tomás, M., Martorell, S., Escalona, J.-M., Pou, A., Fuentes, S., et al. (2015). Improving water use efficiency of vineyards in semi-arid regions. A review. *Agron. Sustain. Dev.* 35, 499–517. doi: 10.1007/s13593-014-0280-z

Minasny, B., Malone, B. P., McBratney, A. B., Angers, D. A., Arrouays, D., Chambers, A., et al. (2017). Soil carbon 4 per mille. *Geoderma* 292, 59–86. doi: 10.1016/j.geoderma.2017.01.002

Mirás-Avalos, J. M., Buesa, I., Llacer, E., Jiménez-Bello, M. A., Risco, D., Castel, J. R., et al. (2017). Water versus source–sink relationships in a semiarid tempranillo vineyard: vine performance and fruit composition. *Am. J. Enol. Vitic.* 68, 11–22. doi: 10.5344/ajev.2016.16026

Mondini, C., Fornasier, F., Sinicco, T., Sivilotti, P., Gaiotti, F., and Mosetti, D. (2018). Organic amendment effectively recovers soil functionality in degraded vineyards. *Eur. J. Agron.* 101, 210–221. doi: 10.1016/j.eja.2018.10.002

Monteiro, A., and Lopes, C. M. (2007). Influence of cover crop on water use and performance of vineyard in Mediterranean Portugal. *Agric. Ecosyst. Environ.* 121, 336–342. doi: 10.1016/j.agee.2006.11.016

Morlat, R. (2008). Long-term additions of organic amendments in a loire valley vineyard on a calcareous sandy soil. II. Effects on root system, growth, grape yield, and foliar nutrient status of a cabernet franc vine. *Am. J. Enol. Vitic.* 59, 364–374.

Morlat, R., and Chaussod, R. (2008). Long-term additions of organic amendments in a loire valley vineyard. I. Effects on properties of a calcareous sandy soil. *Am. J. Enol. Vitic.* 59, 353–363.

Morlat, R., and Symoneaux, R. (2008). Long-term additions of organic amendments in a loire valley vineyard on a calcareous sandy soil. III. Effects on fruit composition and chemical and sensory characteristics of cabernet franc wine. *Am. J. Enol. Vitic.* 59, 375–386.

Mpelasoka, B. S., Schachtman, D. P., Treeby, M. T., and Thomas, M. R. (2003). A review of potassium nutrition in grapevines with special emphasis on berry accumulation. *Aust. J. Grape Wine Res.* 9, 154–168. doi: 10.1111/j.1755-0238.2003.tb00265.x

Mugnai, S., Masi, E., Azzarello, E., and Mancuso, S. (2012). Influence of long-term application of green waste compost on soil characteristics and growth, yield and quality of grape (*Vitis vinifera* L.). *Compost Sci. Util.* 20, 29–33. doi: 10.1080/1065657X.2012.10737019

Muscas, E., Cocco, A., Mercenaro, L., Cabras, M., Lentini, A., Porqueddu, C., et al. (2017). Effects of vineyard floor cover crops on grapevine vigor, yield, and fruit quality, and the development of the vine mealybug under a Mediterranean climate. *Agric. Ecosyst. Environ.* 237, 203–212. doi: 10.1016/j.agee.2016.12.035

Nendel, C., and Reuter, S. (2007). Soil biology and nitrogen dynamics of vineyard soils as affected by a mature biowaste compost application. *Compost Sci. Util.* 15, 70–77. doi: 10.1080/1065657X.2007.10702315

Novara, A., Cerdà, A., and Gristina, L. (2018). Sustainable vineyard floor management: an equilibrium between water consumption and soil conservation. *Curr. Opin. Environ. Sci. Health* 5, 33–37. doi: 10.1016/j.coesh.2018.04.005

Novara, A., Minacapilli, M., Santoro, A., Rodrigo-Comino, J., Carrubba, A., Sarno, M., et al. (2019). Real cover crops contribution to soil organic carbon sequestration in sloping vineyard. *Sci. Total Environ.* 652, 300–306. doi: 10.1016/j.scitotenv.2018.10.247

Olness, A., and Archer, D. (2005). Effect of organic carbon on available water in soil. *Soil Sci.* 170, 90–101. doi: 10.1097/00010694-200502000-00002

Paustian, K., Lehmann, J., Ogle, S., Reay, D., Robertson, G. P., and Smith, P. (2016). Climate-smart soils. *Nature* 532, 49–57. doi: 10.1038/nature17174

Pepi, S., Sansone, L., Chicca, M., and Vaccaro, C. (2017). Relationship among geochemical elements in soil and grapes as terroir fingerprintings in *Vitis vinifera* L. cv. "Glera.". *Geochemistry* 77, 121–130. doi: 10.1016/j.chemer.2017.01.003

Peregrina, F., Larrieta, C., Colina, M., Mariscal-Sancho, I., Martín, I., Martínez-Vidaurre, J. M., et al. (2012). Spent mushroom substrates influence soil quality and nitrogen availability in a semiarid vineyard soil. *Soil Sci. Soc. Am. J.* 76, 1655–1666. doi: 10.2136/sssaj2012.0018

Pérès, G., Cluzeau, D., Curmi, P., and Hallaire, V. (1998). Earthworm activity and soil structure changes due to organic enrichments in vineyard systems. *Biol. Fertil. Soils* 27, 417–424. doi: 10.1007/s003740050452

Pérez-Álvarez, E. P., Pérez-Sotés, J. L., García-Escudero, E., and Peregrina, F. (2013). Cover crop short-term effects on soil no 3 - -n availability, nitrogen nutritional status, yield, and must quality in a calcareous vineyard of the AOC Rioja, Spain. *Commun. Soil Sci. Plant Anal.* 44, 711–721. doi: 10.1080/00103624.2013.748122

Pérez-Bermúdez, P., Olmo, M., Gil, J., García-Férriz, L., Olmo, C., Boluda, R., et al. (2016). Cover crops and pruning in Bobal and Tempranillo vineyards have little influence on grapevine nutrition. *Sci. Agric.* 73, 260–265. doi: 10.1590/0103-9016-2015-0027

Pinamonti, F. (1998). Compost mulch effects on soil fertility, nutritional status and performance of grapevine. *Nutr. Cycl. Agroecosyst.* 51, 239–248. doi: 10.1023/A:1009701323580

Pomarici, E. (2016). Recent trends in the international wine market and arising research questions. *Wine Econ. Policy* 5, 1–3. doi: 10.1016/j.wep.2016.06.001

Ramírez-García, J., Carrillo, J. M., Ruiz, M., Alonso-Ayuso, M., and Quemada, M. (2015). Multicriteria decision analysis applied to cover crop species and cultivars selection. *Field Crops Res.* 175, 106–115. doi: 10.1016/j.fcr.2015.02.008

Ramos, M. C. (2017). Effects of compost amendment on the available soil water and grape yield in vineyards planted after land levelling. *Agric. Water Manag.* 191, 67–76. doi: 10.1016/j.agwat.2017.05.013

Ramos, M. C., and López-Acevedo, M. (2004). Zinc levels in vineyard soils from the Alt Penedès-Anoia region (NE Spain) after compost application. *Adv. Environ. Res.* 8, 687–696. doi: 10.1016/S1093-0191(03)00041-8

Reynard, J.-S., Zufferey, V., Nicol, G.-C., and Murisier, F. (2011). Soil parameters impact the vine-fruit-wine continuum by altering vine nitrogen status. *OENO One* 45, 211–221. doi: 10.20870/oeno-one.2011.45.4.1502

Rockström, J., Steffen, W., Noone, K., Persson, Å., Chapin, F. S., Lambin, E. F., et al. (2009). A safe operating space for humanity. *Nature* 461, 472–475. doi: 10.1038/461472a

Rodrigo-Comino, J. (2018). Five decades of soil erosion research in "terroir". The State-of-the-Art. *Earth Sci. Rev.* 179, 436–447. doi: 10.1016/j.earscirev.2018.02.014

Rodrigo-Comino, J., Keesstra, S., and Cerdà, A. (2018). Soil erosion as an environmental concern in vineyards. The case study of Celler del Roure, Eastern Spain, by Means of Rainfall Simulation Experiments. *Beverages* 4:31. doi: 10.3390/beverages4020031

Ros, M. (2003). Soil microbial activity after restoration of a semiarid soil by organic amendments. *Soil Biol. Biochem.* 35, 463–469. doi: 10.1016/S0038-0717(02)00298-5

Ross, K. (2016). Preparing for an uncertain future with climate smart agriculture. *Calif. Agric.* 70, 4–5. doi: 10.3733/ca.v070n01p4

Rubio, R., Pérez-Murcia, M. D., Agulló, E., Bustamante, M. A., Sánchez, C., Paredes, C., et al. (2013). Recycling of agro-food wastes into vineyards by composting: agronomic validation in field conditions. *Commun. Soil Sci. Plant Anal.* 44, 502–516. doi: 10.1080/00103624.2013.744152

Ruiz-Colmenero, M., Bienes, R., Eldridge, D. J., and Marques, M. J. (2013). Vegetation cover reduces erosion and enhances soil organic carbon in a vineyard in the central Spain. *Catena* 104, 153–160. doi: 10.1016/j.catena.2012.11.007

Salomé, C., Coll, P., Lardo, E., Metay, A., Villenave, C., Marsden, C., et al. (2016). The soil quality concept as a framework to assess management practices in vulnerable agroecosystems: a case study in Mediterranean vineyards. *Ecol. Indic.* 61, 456–465. doi: 10.1016/j.ecolind.2015.09.047

Scandellari, F., Caruso, G., Liguori, G., Meggio, F., Palese, A. M., Zanotelli, D., et al. (2016). A survey of carbon sequestration potential of orchards and vineyards in Italy. *Eur. J. Hortic. Sci.* 81, 106–114. doi: 10.17660/eJHS.2016/81.2.4

Schäufele, I., and Hamm, U. (2017). Consumers' perceptions, preferences and willingness-to-pay for wine with sustainability characteristics: a review. *J. Clean. Prod.* 147, 379–394. doi: 10.1016/j.jclepro.2017.01.118

Schreiner, R. P., and Osborne, J. (2018). Defining phosphorus requirements for pinot noir grapevines. *Am. J. Enol. Vitic.* 69, 351–359. doi: 10.5344/ajev.2018.18016

Schulte, R. P. O., Creamer, R. E., Donnellan, T., Farrelly, N., Fealy, R., O'Donoghue, C., et al. (2014). Functional land management: a framework for managing soil-based ecosystem services for the sustainable intensification of agriculture. *Environ. Sci. Policy* 38, 45–58. doi: 10.1016/j.envsci.2013.10.002

Schütte, R., and Bergmann, H. (2019). The attitudes of French and Spanish winegrowers towards the use of cover crops in vineyards. *J. Wine Res.* 30, 107–121. doi: 10.1080/09571264.2019.1568975

Shackelford, G. E., Kelsey, R., and Dicks, L. V. (2019). Effects of cover crops on multiple ecosystem services: ten meta-analyses of data from arable farmland in California and the Mediterranean. *Land Use Policy* 88, 1–10. doi: 10.1016/j.landusepol.2019.104204

Six, J., Elliott, E. T., and Paustian, K. (2000). Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. *Soil Biol. Biochem.* 32, 2099–2103. doi: 10.1016/S0038-0717(00)00179-6

Skinner, P. W., Cook, J. A., and Matthews, M. A. (1988). Phosphorus fertilizer applications under phosphorus-limited. *Vitis* 27, 95–109.

Sokol, N. W., and Bradford, M. A. (2019). Microbial formation of stable soil carbon is more efficient from belowground than aboveground input. *Nat. Geosci.* 12, 46–53. doi: 10.1038/s41561-018-0258-6

Sokol, N. W., Kuebbing, S. E., Karlsen-Ayala, E., and Bradford, M. A. (2019). Evidence for the primacy of living root inputs, not root or shoot litter, in forming soil organic carbon. *New Phytol.* 221, 233–246. doi: 10.1111/nph.15361

Steenwerth, K. L., McElrone, A. J., Calderon-Orellana, A., Hanifin, R. C., Storm, C., Collatz, W., et al. (2013). Cover crops and tillage in a mature merlot vineyard show few effects on grapevines. *Am. J. Enol. Vitic.* 64, 515–521. doi: 10.5344/ajev.2013.12119

Steenwerth, K., and Belina, K. M. (2008a). Cover crops and cultivation: impacts on soil N dynamics and microbiological function in a Mediterranean vineyard agroecosystem. *Appl. Soil Ecol.* 40, 370–380. doi: 10.1016/j.apsoil.2008.06.004

Steenwerth, K., and Belina, K. M. (2008b). Cover crops enhance soil organic matter, carbon dynamics and microbiological function in a vineyard agroecosystem. *Appl. Soil Ecol.* 40, 359–369. doi: 10.1016/j.apsoil.2008.06.006

Stewart, R. D., Jian, J., Gyawali, A. J., Thomason, W. E., Badgley, B. D., Reiter, M. S., et al. (2018). What we talk about when we talk about soil health. *Agric. Environ. Lett.* 3, 1–5. doi: 10.2134/ael2018.06.0033

Suddick, E. C., Ngugi, M. K., Paustian, K., and Six, J. (2013). Monitoring soil carbon will prepare growers for a carbon trading system. *Calif. Agric.* 67, 162–171. doi: 10.3733/ca.v067n03p162

Sweet, R. M., and Schreiner, R. P. (2010). Alleyway cover crops have little influence on pinot noir grapevines (*Vitis vinifera* L.) in Two Western Oregon Vineyards. *Am. J. Enol. Vitic.* 61, 240–252.

Teasdale, J. R., and Mohler, C. L. (1993). Light transmittance, soil temperature, and soil moisture under residue of hairy vetch and rye. *Agron. J.* 85, 673–680. doi: 10.2134/agronj1993.00021962008500030029x

Tesic, D., Keller, M., and Hutton, R. J. (2007). Influence of vineyard floor management practices on grapevine vegetative growth, yield, and fruit composition. *Am. J. Enol. Vitic.* 58, 1–11.

Van Leeuwen, C., Roby, J.-P., and De Rességuier, L. (2018). Soil-related terroir factors: a review. *OENO One* 52, 173–188. doi: 10.20870/oeno-one.2018.52.2.2208

Vandenkoornhuyse, P., Quaiser, A., Duhamel, M., Le Van, A., and Dufresne, A. (2015). The importance of the microbiome of the plant holobiont. *New Phytol.* 206, 1196–1206. doi: 10.1111/nph.13312

Vaudour, E., Costantini, E., Jones, G. V., and Mocali, S. (2015). An overview of the recent approaches to terroir functional modelling, footprinting and zoning. *Soil* 1, 287–312. doi: 10.5194/soil-1-287-2015

Vereecken, H. (1995). Estimating the unsaturated hydraulic conductivity from theoretical models using simple soil properties. *Geoderma* 65, 81–92. doi: 10.1016/0016-7061(95)92543-X

Verhoeven, E., Decock, C., Garland, G., and Lazcano, C. (2019). Vineyard nitrous oxide (N_2O) emissions. *Wine Business Monthly*, 196–205.

Verhoeven, E., Pereira, E., Decock, C., Garland, G., Kennedy, T., Suddick, E., et al. (2017). N 2 O emissions from California farmlands: a review. *Calif. Agric.* 71, 148–159. doi: 10.3733/ca.2017a0026

Versari, A., Laurie, V. F., Ricci, A., Laghi, L., and Parpinello, G. P. (2014). Progress in authentication, typification and traceability of grapes and wines by chemometric approaches. *Food Res. Int.* 60, 2–18. doi: 10.1016/j.foodres.2014.02.007

Vicente-Vicente, J. L., García-Ruiz, R., Francaviglia, R., Aguilera, E., and Smith, P. (2016). Soil carbon sequestration rates under Mediterranean woody crops using recommended management practices: a meta-analysis. *Agric. Ecosyst. Environ.* 235, 204–214. doi: 10.1016/j.agee.2016.10.024

Vogel, H.-J., Wollschläger, U., Helming, K., Heinrich, U., Willms, M., Wiesmeier, M., et al. (2019). "Assessment of soil functions affected by soil management," in *Atlas of Ecosystem Services*, eds M. Schröter, A. Bonn, S. Klotz, R. Seppelt, and C. Baessler (Cham: Springer), 77–82. doi: 10.1007/978-3-319-96229-0_13

Wheeler, S. J., and Pickering, G. J. (2003). Optimizing grape quality through soil management practices. *Food Agric. Environ.* 1, 190–197.

White, R. E. (2015). *Understanding Vineyard Soils*. Oxford: Oxford University Press.

White, R. E., Balachandra, L., Edis, R., and Chen, D. (2007). The soil component of terroir. *OENO One* 41, 9–18. doi: 10.20870/oeno-one.2007.41.1.860

Wienhold, B. J., Andrews, S. S., and Karlen, D. L. (2004). Soil quality: a review of the science and experiences in the USA. *Environ. Geochem. Health* 26, 89–95. doi: 10.1023/B:EGAH.0000039571.59640.3c

Williams, L. E. (1999). *Fertilizer Use Efficiency and Influence of Rootstocks on Uptake and Accumulation of Nutrients in Wine Grapes Grown in the Coastal Valleys of California*. Davis, CA: UC Davis Department of Viticulture and Enology.

Wilson, S. G., Lambert, J.-J., and Dahlgren, R. A. (2016). Seasonal phosphorus dynamics in a volcanic soil of Northern California. *Soil Sci. Soc. Am. J.* 80, 1222–1230. doi: 10.2136/sssaj2016.02.0028

Wilson, S. G., Lambert, J.-J., Nanzyo, M., and Dahlgren, R. A. (2017). Soil genesis and mineralogy across a volcanic lithosequence. *Geoderma* 285, 301–312. doi: 10.1016/j.geoderma.2016.09.013

Winter, S., Bauer, T., Strauss, P., Kratschmer, S., Paredes, D., Popescu, D., et al. (2018). Effects of vegetation management intensity on biodiversity and ecosystem services in vineyards: a meta-analysis. *J. Appl. Ecol.* 55, 2484–2495. doi: 10.1111/1365-2664.13124

Wolff, M. W., Alsina, M. M., Stockert, C. M., Khalsa, S. D. S., and Smart, D. R. (2018). Minimum tillage of a cover crop lowers net GWP and sequesters soil carbon in a California vineyard. *Soil Tillage Res.* 175, 244–254. doi: 10.1016/j.still.2017.06.003

Zarraonaindia, I., Owens, S. M., Weisenhorn, P., West, K., Hampton-Marcell, J., Lax, S., et al. (2015). The soil microbiome influences grapevine-associated microbiota. *mBio* 6:e02527-14. doi: 10.1128/mBio.02527-14

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Spatial Variability of Soil and Plant Water Status and Their Cascading Effects on Grapevine Physiology Are Linked to Berry and Wine Chemistry

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The relationships between differences in plant water status, induced by spatial variability in soil texture, and the changes in berry and wine composition were investigated in an irrigated Cabernet Sauvignon (*Vitis vinefera* L.) vineyard for 2 years. A stratified and an equidistant grid were overlaid on the vineyard to characterize the soil texture by proximal sensing, soil sampling, and grapevine physiological and berry chemical development. Based on the mid-day stem water potential (Ψ_{stem}) integrals, the vineyard was divided into two functional homogenous zones: Zone 1 with higher water stress and Zone 2 with lower water. Zone 1 consistently had lower Ψ_{stem}, net carbon assimilation, and stomatal conductance in both years. Berry weight and titratable acidity were lower in Zone 1 at harvest. Zone 2 reached 26 and 24°Bx total soluble solids (TSS) at harvest in Years 1 and 2, respectively, with higher TSS values of 30 and 27°Bx in Zone 1. Ravaz index did not vary spatially. Fruits were harvested differentially in both years and vinified separately from the two zones. In Year 1, all berry skin anthocyanin derivatives, tri-, di- hydroxylated, and total anthocyanins concentrations were higher in Zone 2. However, in Year 2, only malvidin, tri-hydroxylated, and total anthocyanins were higher in Zone 1. There were no differences in wine flavonoids in Year 2 when harvest commenced earlier. In both years, Ψ_{stem}, berry weight, and TSS were directly related to soil bulk electrical conductivity (EC). Our results indicated vineyard variability stemmed from soil texture that affected long-term plant water status which does not affect spatial variability of Ravaz Index. In conclusion, our work provides fundamental knowledge about the applicability of soil bulk EC sensing in the vineyards, and its potential directional utilization by connecting proximal soil sensing to spatial distribution of whole-plant physiological performance together with berry and wine chemistry.

Keywords: viticulture, plant water status, soil electrical conductivity, spatial variability, flavonoids, wine

INTRODUCTION

There is natural spatial variability present in vineyards due to the variations in soil characteristics and topography (Brillante et al., 2016a). Soil characteristics are too complex to be thoroughly surveyed effortlessly. With traditional destructive methods, it is difficult to obtain enough comprehensive information from the soil pits at the field scale. These soil characteristics may

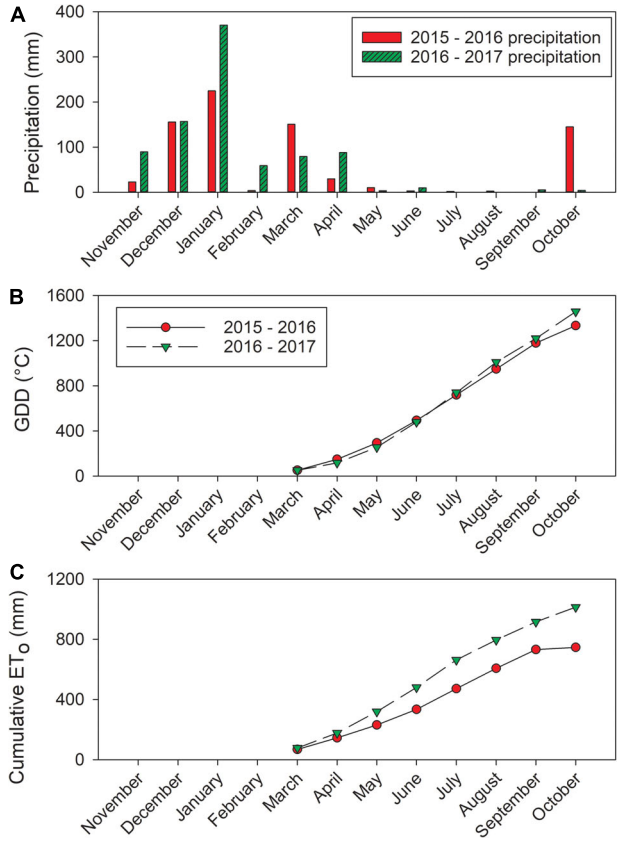

FIGURE 1 | Weather data acquired from California Irrigation Management Information System (CIMIS) station (#86, Santa Rosa, CA). **(A)** Precipitation, **(B)** calculated GDD, and **(C)** cumulative ET_O.

directly affect the water availability for grapevines, which eventually determine the physiological performance of the plants (Brillante et al., 2015, 2016a). However, there is no variable management practices currently available to accommodate the natural spatial variability. Thus, the spatial variability derived from vineyard soils will inevitably be expressed in the whole plant physiology at the cost of homogeneity of vineyard productivity and quality. We previously reported the spatial variation of mid-day stem water potential affecting grapevine carbon assimilation and stomatal conductance of grapevine (Brillante et al., 2017; Yu and Kurtural, 2020). The resultant variations in whole-plant physiology were associated to flavonoid composition and concentration at the farm gate. However, there is a lack of information about the effects on the chemical composition in the final wine, which would ultimately determine wine quality as perceived by consumers.

Georeferenced proximal sensing tools can capture the spatial and temporal variability in vineyards, making it possible to supervise and manage variations at the field scale (Bramley et al., 2011c; Matese et al., 2015). Previous studies showed that soil bulk electrical conductivity (EC) may be used to evaluate many soil attributes, including soil moisture content, salinity, and texture (Brillante et al., 2014; Su et al., 2014). Soil electromagnetic induction (EMI) sensing has been used in precision agriculture to acquire soil bulk EC at the field scale due to its non-invasive and prompt attributes (Bramley et al., 2011a; Rodríguez-Pérez et al., 2011). Although research had been conducted on the relationships between soil electrical properties with plant water status, they were mostly point measurements and the results were rarely interpolated to whole fields. There were only a few studies that investigated the EMI sensing and soil-plant water relationships over a vineyard (Bonfante et al., 2015). Previous research suggested that the connection between soil water content and soil bulk EC could have relied on specific soil profiles, and needed to include soil physical and chemical properties to complete this connection (Brillante et al., 2014, 2016a). Nevertheless, there is evidence that soil bulk EC may still be useful not only to identify the variability in soil, but also in the plant response affected by vineyard soils such as yield, plant physiology, and grape berry chemistry (Bramley et al., 2011a; Tagarakis et al., 2013).

Plant available water is a determinant factor on grapevine physiology, together with nitrogen availability in semi-arid regions (Smart and Coombe, 1983). Wine grapes are usually grown under a moderate degree of water deficits as yields were optimized at 80% of crop evapotranspiration demand with sustained deficit irrigation (Williams, 2012). Water deficits would limit leaf stomatal conductance and carbon assimilation rate that sustain grapevines' vegetative and reproductive growth and development (Escalona et al., 2015). When grapevines are under water deficits, carbohydrates repartitioned into the smaller berries would enhance berry soluble solids content (Escalona et al., 2015). Sucrose and fructose, which are the major components of total soluble solids (TSS) in grape berry, can act as a signaling factor to stimulate anthocyanin accumulation (Dai et al., 2014). The effects on grapevine physiology and berry composition also depend on the phenological stages they occur and how severe and prolonged the water deficits are (Intrigliolo and Castel, 2010).

Flavonoids are the most critical compounds dictating many qualitative traits in both grape berries and wine (Lorrain et al., 2013). The variations in environmental factors could alter the concentration and biosynthesis of flavonoids and can be extrapolated spatially within the same vineyard, including water deficits (Castellarin et al., 2007b), solar radiation (Martínez-Lüscher et al., 2019), and air temperature (Spayd et al., 2002). Among flavonoid compounds, anthocyanins are responsible for the color of berry skin as well as wine (Intrigliolo and Castel, 2010). Moderate water deficits during growing season can increase anthocyanin concentration in berry skin and wine (Cortell et al., 2007). However, water deficits can impair plant temperature regulation through evaporative cooling (Tombesi et al., 2015). They may also inhibit berry growth by limiting berry size and altering berry skin weight (Castellarin et al., 2007a; Santesteban et al., 2011). Thus, in some cases it may be uncertain if water deficit promotes anthocyanins biosynthesis or reduces berry growth, or contributes to anthocyanin degradation (Petrussa et al., 2013). Applying water deficit on grapevines can contribute to greater proportion in tri-hydroxylated over di-hydroxylated anthocyanins due to the up-regulation of F3′5′H

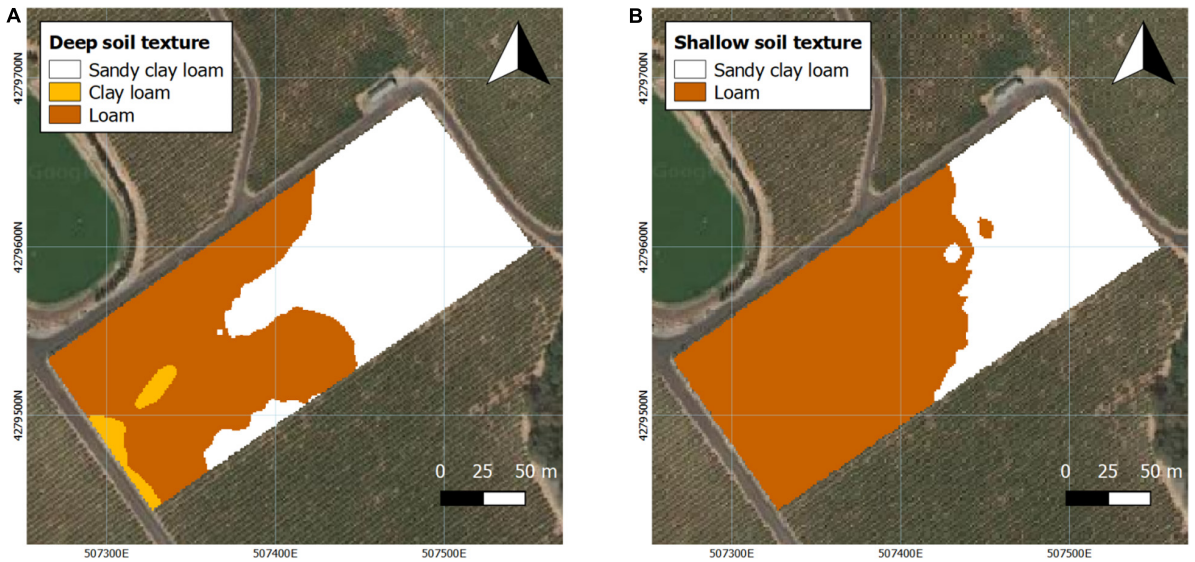

FIGURE 2 | Interpolated projection of soil texture at two depths assessed in 2016 and 2017. **(A)** Deep soil (0.75–1.5 m), **(B)** shallow soil (0–0.75 m). Coordinate system: WGS 1984 UTM Zone 10N.

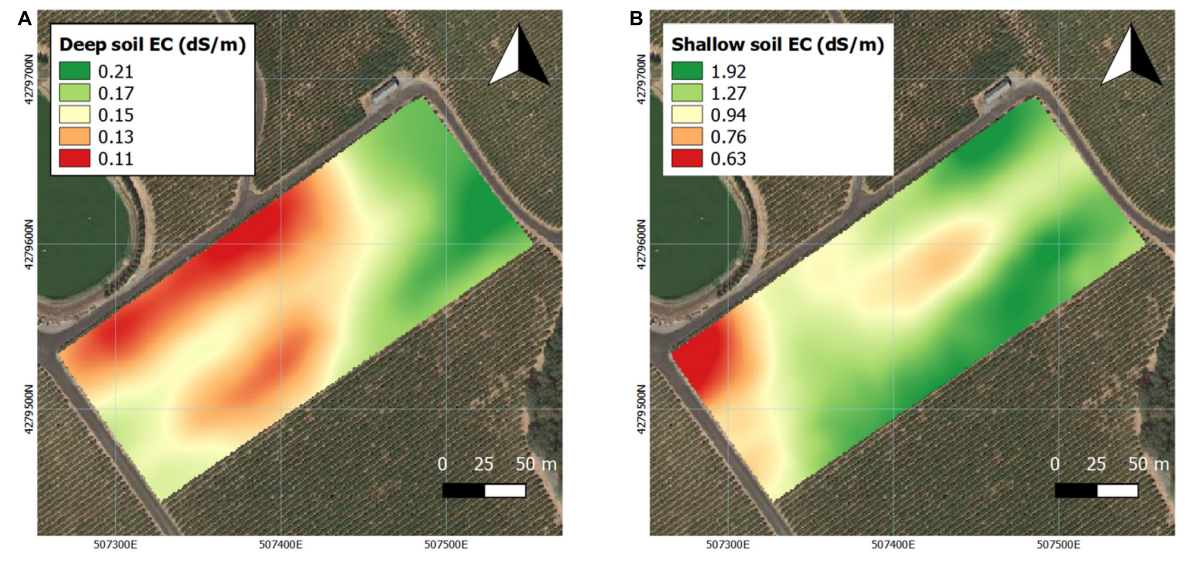

FIGURE 3 | Interpolation soil electrical conductivity (EC) in two depths assessed by EM38 in 2016 and 2017. **(A)** Deep soil (0–1.5 m), **(B)** shallow soil (0–0.75 m). Coordinate system: WGS 1984 UTM Zone 10N.

(Castellarin et al., 2007b; Martínez-Lüscher et al., 2014). Another major class in flavonoids, proanthocyanidins, are polymers of flavan-3-ol monomers and they contributes mainly toward astringency (tactile sensation) or bitterness (taste) in wine (Gonzalo-Diago et al., 2013). Compared to anthocyanins, water deficits showed mild effects on proanthocyanidins (Bucchetti et al., 2011). However, water deficits with great severity can still alter the concentration and composition of proanthocyanidins in both berries and wine (Ollé et al., 2011).

Selective harvest is one of the targeted management strategies to minimize the spatial variation in berry chemistry in vineyards (Scarlett et al., 2014). By differentially harvesting or segregating the fruits into batches prior to vinification, the berry composition can be artificially set at a more uniform stage with minimal variations (Bramley et al., 2011b). In our previous work, we reported the use of plant water status to determine the spatial variation of grape berry flavonoids (Brillante et al., 2017). The goal of this study was to deduce if the spatial variability of soil bulk EC and differences in soil texture can be related to plant physiology and grape and wine composition. The specific objective of the study was to determine if the spatial variability of proximally sensed vineyard soil bulk EC would affect plant

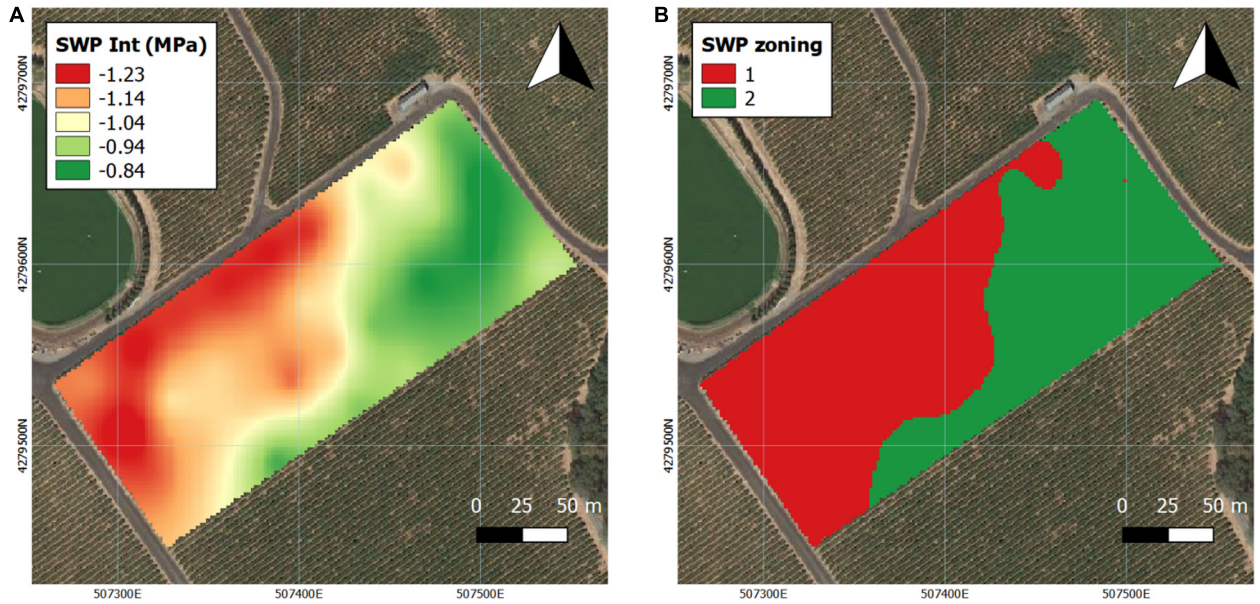

FIGURE 4 | Interpolation maps of plant water status (expressed as stem water potential Ψ_{stem}) and *k*-means clustering, delineating the vineyard into two zones in 2017. **(A)** Ψ_{stem} kriging maps, **(B)** *k*-means clustering maps. Coordinate system: WGS 1984 UTM Zone 10N.

water status, and if this relation would affect leaf gas exchange, components of yield, berry composition, and flavonoids in both berries and wine.

MATERIALS AND METHODS

Vineyard Site, Plant Materials, and Weather

The study was conducted in a commercial vineyard in 2016 and 2017 with Cabernet Sauvignon (*Vitis vinifera* L.) grapevines grafted on 110R (*Vitis berlandieri* Planch. × *Vitis rupestris* Scheele) located in Healdsburg, CA, United States. In this vineyard, grapevines were planted at 1.83 m × 3.35 m (vine × row). The grapevines were trained to a high-quadrilateral, horizontally split trellis with two bilateral cordons. They were spur pruned with two buds per spur, and seven spurs per meter of the cordon. Irrigation was applied uniformly with a drip irrigation system, starting at fruit-set to the end of veraison at 50% ET_c. There were two emitters per grapevine, delivering 3.8 $L{\cdot}h^{-1}$ of water. Weather data was obtained from the California Irrigation Management Information System (CIMIS) station (#86, Santa Rosa, CA, United States) to measure precipitation, air temperature, and reference evapotranspiration (**Figure 1**).

Experimental Design

An equidistant 33 m × 33 m grid with 35 experimental units was used for on-site measurements and berry samplings. Each experimental unit consisted of five plants. The locations of each central plant in these five plant experimental units were registered as the grid nodes with a GPS (Yuma 2, Trimble Inc., Sunnyvale, CA, United States), wirelessly connected to a Trimble Pro 6T DGNSS receiver (Trimble Inc., Sunnyvale, CA, United States).

Vineyard Soil Property Assessment

Soil bulk EC was assessed with EM38 (Geonics Ltd., Mississauga, ON, Canada) in 2016 when the vineyard soil was at field capacity condition. Both vertical dipole mode and horizontal dipole mode were used to assess EC at two depths, including deep soil (0–1.50 m) and shallow soil (0–0.75 m). The instrument was calibrated according to manufacturer instructions. The device was placed on a PVC sled and driven through the vineyard with an all-terrain vehicle along the inter-rows. A distance of approximately 0.5 m from the vehicle to the device was maintained to avoid interference with the vehicle. A stratified grid was used to collect soil samples corresponding to the two depths at which we measured soil bulk EC. Soil texture was assessed according to the soil analysis method: hydrometer analysis (S – 14.10) in the North American Proficiency Testing (NAPT) program.

Grapevine Physiology Assessments

Plant water status was assessed biweekly by midday stem water potential (Ψ_{stem}) measurements. The measurements for Ψ_{stem} in 2016 were previously described in 2016. In 2017, Ψ_{stem} was assessed on 27 June, 13 July, 27 July, 8 August, 24 August, 8 September, and 19 September. The measurements were conducted at solar noon from 12:00 to 14:30 h. Three leaves from main shoot axes in the shade were selected and concealed in pinch-sealed Mylar® bags for 2 h prior to the measurements. A pressure chamber (Model 615D, PMS Instrument Company, Albany, OR, United States) was used to take the measurements. To summarize the season-long plant water status, Ψ_{stem} integrals were calculated by using natural

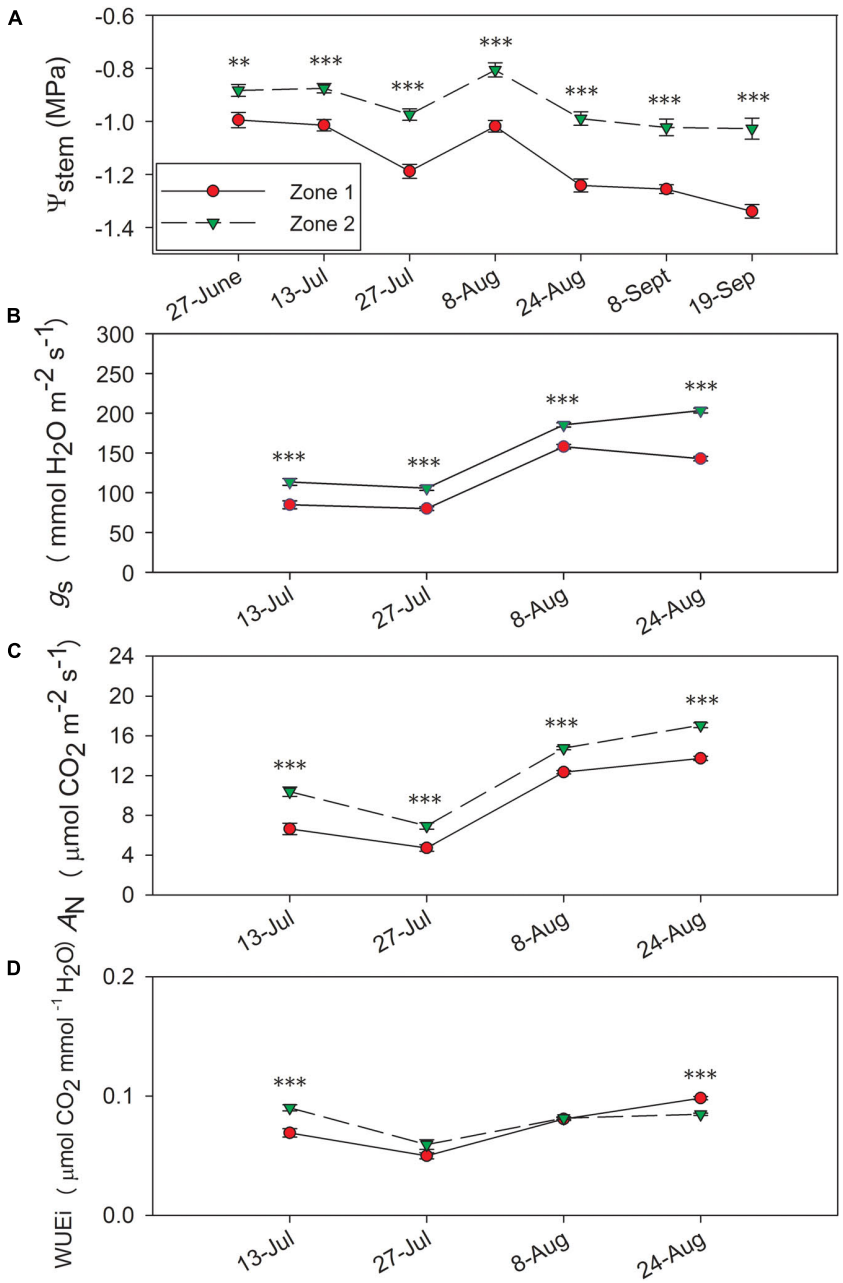

FIGURE 5 | Progression of stem water potential Ψ_{stem} and gas exchange between the two water status zones in 2017. **(A)** Ψ_{stem}, **(B)** stomatal conductance, g_s, **(C)** net carbon assimilation, A_N, **(D)** intrinsic water use efficiency, WUEi. Error bars represent standard error of the mean. Asterisks represents significant levels p: ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$.

cubic splines, and then normalized by the number of days elapsed from the first measurement to the last.

Leaf gas exchange measurements were taken biweekly by using a portable infrared gas analyzer CIRAS-3 (PP Systems, Amesbury, MA, United States). The measurements for leaf gas exchange in 2016 were previously described in 2016. In 2017, leaf gas exchange was assessed on 13 July, 27 July, 8 August, and 24 August. The gas analyzer was set to a relative humidity of 40% and the reference CO_2 concentration of 400 μmol $CO_2 \cdot mol^{-1}$. Three sun-exposed leaves from the main shoot axis were measured in each vine, and the three middle vines were selected in each experimental unit. Gas exchange measurements were taken when the sunlight was at saturation conditions in both years (average $PAR_i = 1969 \pm 135$ $\mu mol \cdot m^{-2} \cdot s^{-1}$ in 2016, 1884 ± 165 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in 2017).

Yield components were measured on a single harvest day in each season (5 October 2016 and 20 September 2017). The dates were chosen to follow the grower's harvest schedule. The clusters

TABLE 1 | Yield components and berry primary metabolites of Cabernet Sauvignon as separated by water status zoning in Sonoma County, CA in 2016 and 2017[a,b].

		Yield (tons·ha^{-1})	Skin weight (mg)	Berry (no.·m^{-1})	Pruning weight (kg·vine^{-1})	Ravaz index (kg·kg^{-1})	TSS (°Brix) [c]	pH	TA (g·L^{-1}) [d]
2016	Zone 1 ± SE [w]	7.06 ± 0.48	60.54 ± 2.71	2879.42 ± 157.73	2.08 ± 0.05 ±	2.43 ± 0.26	29.88 ± 0.25 a [e]	3.92 ± 0.02 a	5.40 ± 0.11 b
	Zone 2 ± SE	6.70 ± 0.77	55.12 ± 2.76	2529.03 ± 230.84	2.07 ± 0.14	2.31 ± 0.45	26.32 ± 0.38 b	3.75 ± 0.01 b	6.01 ± 0.12 a
	p-value	ns	ns	ns	ns	ns	0.027	<0.0001	0.001
2017	Zone 1 ± SE	13.66 ± 0.66	62.25 ± 1.80 a	5997.40 ± 341.15	1.66 ± 0.07	5.88 ± 0.37	26.72 ± 0.39 a	3.65 ± 0.03 a	6.53 ± 0.12 b
	Zone 2 ± SE	11.68 ± 1.45	52.98 ± 2.04 b	4408.16 ± 434.27	1.70 ± 0.14	4.92 ± 1.02	23.71 ± 0.40 b	3.58 ± 0.01 b	7.22 ± 0.10 a
	p-value	ns	0.002	ns	ns	ns	<0.0001	0.024	<0.0001
Year		<0.0001	< 0.0001	<0.0001	0.002	0.0001	<0.0001	< 0.0001	<0.0001
Year × Zoning		ns	ns	ns	ns	ns	ns	ns	ns

[a]n = 35, ns: not significant, the data for 2016 in this table was partially published in Brillante et al. (2017), courtesy of American Chemical Society. [b]Except skin weight, components of yield were expressed as numbers or weight per meter of vineyard row. [c]TSS, total soluble solids; TA, titratable acidity. [d]Zone 1: lower plant water status zone, Zone 2: higher plant water status zone. Numbers in the column were expressed as their means ± standard error of the mean. [e]Different letters indicate significant mean separation according to Tukey's HSD test ($p < 0.05$).

from the three middle vines in each experimental unit were harvested, counted, and weighed. Cluster weight was calculated by dividing crop weight by cluster number. A total of 75 berries were randomly selected from the five vines in each experimental unit, and were separated into two subsets of 55 and 20 berries. The first set with 55 berries was used for berry composition analysis. The second set with 20 berries was for measuring berry skin mass and skin flavonoid contents. The average berry weight were assessed from the average weight of the total 75 berries. Pruning weight per vine was collected during the dormant season. Ravaz index was calculated as the ratio of the yield per vine and the pruning weight per vine.

Berry Total Soluble Solids, pH, and Titratable Acidity

Berry samples were taken biweekly throughout each season. In 2016, berry wet chemistry was assessed on 15 July, 28 July, 11 August, 23 August, 1 September, 15 September, and 5 October. In 2017, berry wet chemistry was assessed on 13 July, 27 July, 8 August, 24 August, 7 September, and 20 September. Total soluble solids (TSS, measured as°Brix), pH, and titratable acidity (TA) were analyzed on the must. Berry TSS were measured by a digital refractometer (Atago PR-32, Bellevue, WA, United States). Must pH and TA (expressed as g of tartaric acid per L of must after titration to pH 8.3) were measured with an automated titrator (862 Compact TitroSampler, Metrohm, Switzerland).

Extraction of Skin Flavonoid Compounds

Skins were manually peeled from the 20 berries with a scalpel, and lyophilized by a freeze-drier (Triad Freeze-Dry System, Labconco, Kansas City, MO, United States). Skin tissues were then powderized with a mixing mill (MM400, Retsch, Mammelzen, Germany). For anthocyanin analysis, 50 mg of dry skin powder was weighed and extracted with 1 mL of methanol:water:7 M hydrochloric acid (70:29:1) solution at 4°C overnight. Extracts were centrifuged at 5,000 rpm for 10 min, the supernatants were filtered by PTFE membrane filters (diameter: 13 mm, pore size: 0.45 μm, VWR, Seattle, WA, United States), and transferred into high performance liquid chromatography system (HPLC) vials before injection.

Berry and Wine Flavonoid Analysis

Skin anthocyanins were analyzed by a reversed-phase HPLC (Agilent model 1260, Agilent Technologies, Santa Clara, CA, United States) consisting of a vacuum degasser, an autosampler, a quaternary pump, and a diode array detector with a column heater. A C18 reversed-phase column (LiChrosphere 100 RP-18, 4 × 520 mm^2, 5 μm particle size, Agilent Technologies, Santa Clara, CA, United States) was utilized for analyzing anthocyanins. The mobile phase flow rate was 0.5 mL·min^{-1}, and two mobile phases were used, which included solvent A = 5.5% aqueous formic acid (v/v) and solvent B = 5.5% formic acid in acetonitrile (v/v). The HPLC flow gradient started with 91.5% A with 8.5% B; 87% A with 13% B at 25 min; 82% A with 18% B at 35 min; 62% A with 38% B at 70 mins; 50% A with 50% B at 70.01 min; 30% A with 70% B at 75 min; 91.5% A with 8.5% B from

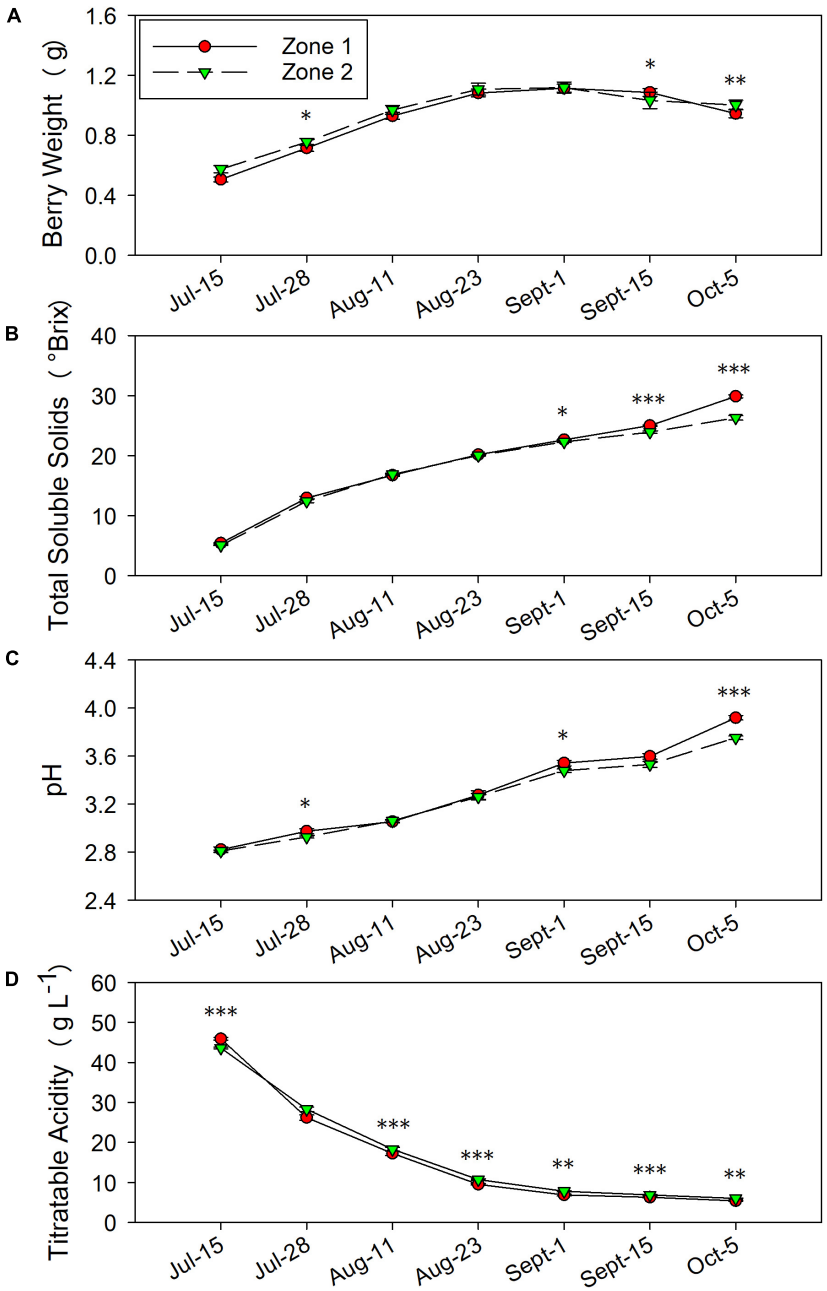

FIGURE 6 | Temporal development of grape berry primary metabolites between the two plant water status zones in 2016. **(A)** Berry weight, **(B)** total soluble solids, **(C)** pH, **(D)** titratable acidity. Error bars represent standard error of the mean. Asterisks represents significant levels p: ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$.

75.01 min to 90 min. The column temperature was maintained at 25°C. Detection of anthocyanins was carried out by the diode array detector at 520 nm. A computer workstation with Agilent OpenLAB (Chemstation edition, version A.02.10) was used for chromatographic analysis.

Wine proanthocyanidin subunits were characterized by acid catalysis in the presence of excess phloroglucinol by reversed-phase HPLC (Agilent model 1100, Agilent Technologies, Santa Clara, CA, United States) (Kennedy and Jones, 2001). 1 mL of wine sample was applied to the Bond Elut C18 OH solid phase extraction cartridges (Agilent Technologies, Santa Clara, CA, United States) to purify wine proanthocyanidins. Eluents were evaporated and resuspended in 1 mL of methanol, and 0.25 mL methanolic extracts were combined with 0.25 mL of phloroglucinolysis reagent (100 $g{\cdot}L^{-1}$ phloroglucinolysis and 20 $g{\cdot}L^{-1}$ ascorbic acid with 0.2 N HCl at methanol). The mixtures were then water bathed at 50°C for 20 min. The reaction was stopped by mixing 200 μL of the sample mixtures with 1 mL

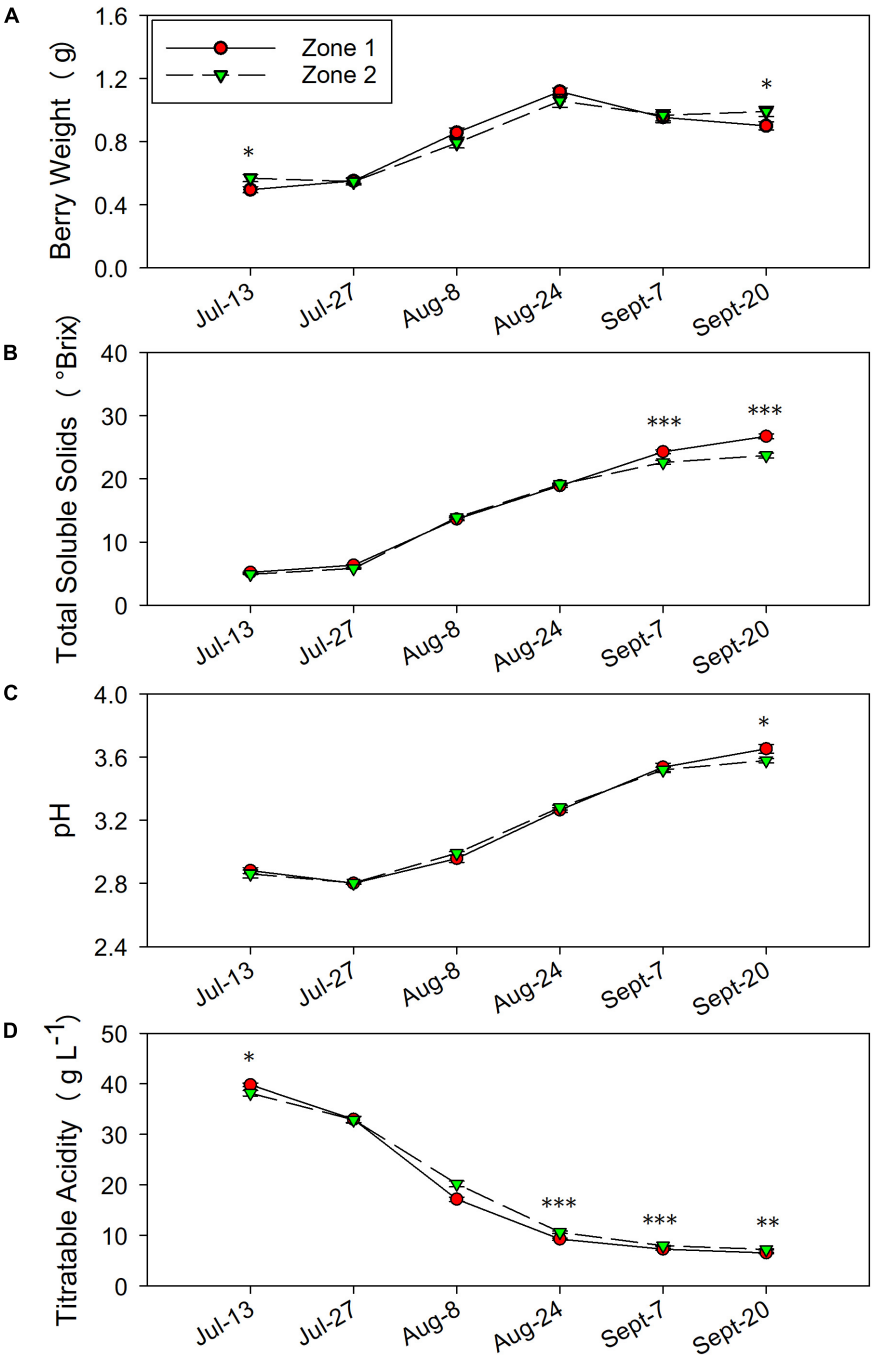

FIGURE 7 | Temporal development of grape berry primary metabolites between the two plant water status zones in 2017. **(A)** Berry weight, **(B)** total soluble solids, **(C)** pH, **(D)** titratable acidity. Error bars represent standard error of the mean. Asterisks represents significant levels *p*: ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$.

of stopping reagent (40 mM aqueous sodium acetate) and then injected into the HPLC. The HPLC column consisted of two Chromolith RP-18e (100 × 4.6 mm^2) columns serially connected and protected by a guard column with the same material (4 × 4 mm^2) from EM Science (Gibbstown, NJ, United States). The mobile phase flow rate was 3.0 $mL{\cdot}min^{-1}$. Two mobile phases were used, which included solvent A = 1% aqueous acetic acid (v/v) and solvent B = 1% acetic acid in acetonitrile (v/v). The HPLC flow gradient started with 97% A with 3% B; 82% A, 18% B at 14 min; 20% A, 80% B at 14.01 min; 97% A, 3% B at 16.01 min until 20 min.

All solvents used in this analysis were of HPLC grade, including acetonitrile, methanol, hydrochloric acid, and formic acid purchased from Fisher Scientific (Santa Clara, CA,

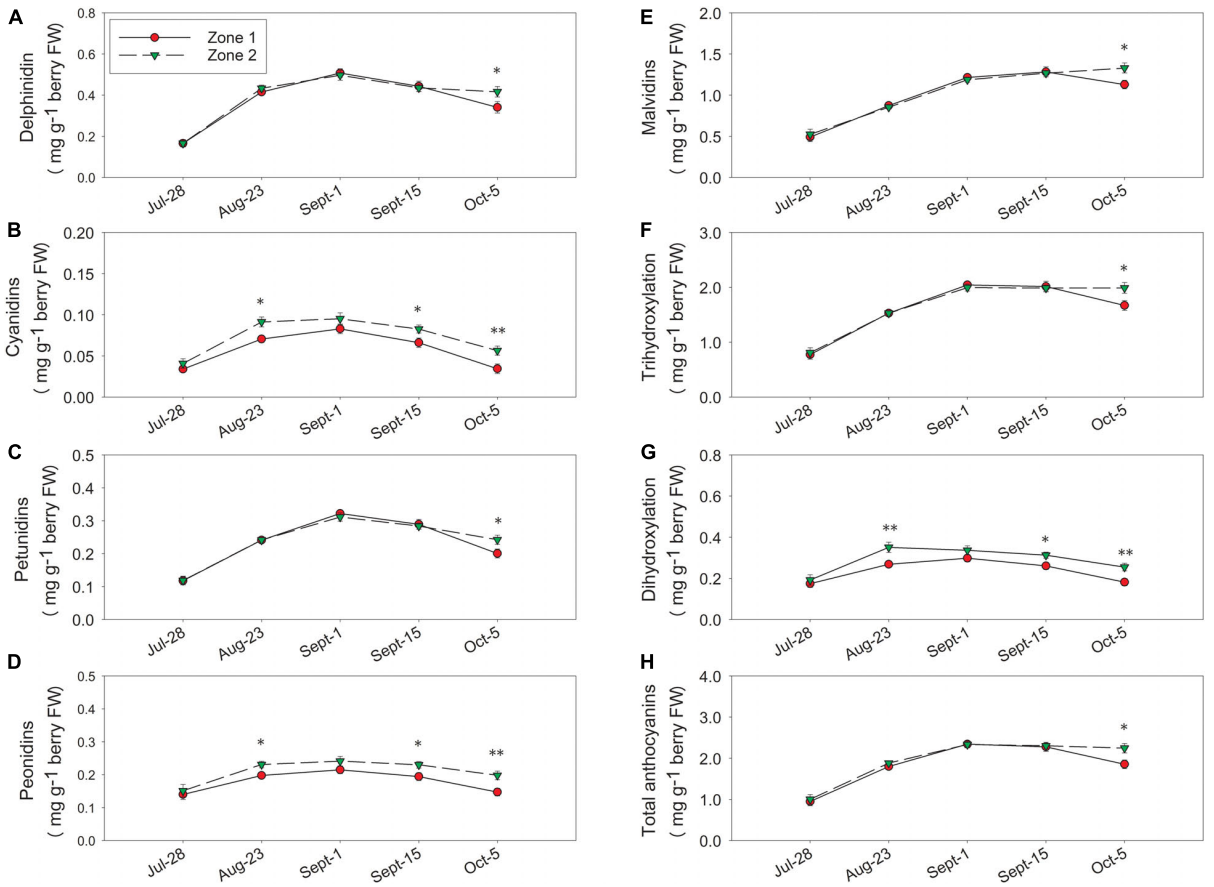

FIGURE 8 | Temporal development of grape berry anthocyanins between the two plant water status zones in 2016. **(A)** Delphinidins, **(B)** cyanidins, **(C)** petunidins, **(D)** peonidins, **(E)** malvidins, **(F)** tri-hydroxylation, **(G)** di-hydroxylation, **(H)** total anthocyanins. Error bars represent standard error of the mean. Asterisks represents significant levels p: ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$.

United States). Standards used for compound identification included malvidin 3-*O*-glucoside, (-)-epicatechin purchased from Extrasynthese (Genay, France). Phloroglucinol was purchased from VWR (Visalia, CA, United States).

Statistical Analysis

Geostatistical analysis was performed in the R language by using package "gstat" 1.1-6 (Pebesma, 2004). The bulk EC data were filtered by Tukey's rule to remove outliers either below the first quartile by 1.5 inter-quartile range or above the third quartile by 1.5 inter-quartile range. To further remove the outliers, the data were filtered by the speed that the vehicle was driving, which was between 3.2 km per hour to 8.0 km per hour. Variograms were assessed by "automap" package 1.0-14 (Hiemstra, 2013), and fitted to perform kriging. The soil bulk EC values were extracted from the location of each experimental unit, these values were further used to perform regression analysis. Kriging and *k*-means clustering on plant physiology variables were performed with the R packages "gstat" and "NbClust," v3.0 (Charrad et al., 2014). Universal kriging was utilized on plant water status because of the existing trend in longitude and latitude. Variograms were assessed by "automap" package 1.0-14 (Hiemstra, 2013), and fitted to perform universal kriging. The vineyard was delineated into two clusters by *k*-means clustering, including Zone 1 with higher water deficit and Zone 2 with lower water deficits. The separation described 78.1% in 2017 of the variability in the plant water status according to the result of between sum of squares/total sum of squares. The resulting maps were organized and displayed by using QGIS software (version 2.14.12, QGIS Development Team). Cluster comparison was analyzed by "raster" package reported as Pearson's Correlation between two cluster maps (Hijmans et al., 2015).

Data were tested for normality by using Shapiro-Wilk's test, and subjected to mean separation by using two-way ANOVA with the package "stats" in RStudio (R Foundation for Statistical Computing, Vienna, Austria) (R Core Team, 2019). Significant statistical differences were determined when p values acquired from ANOVA were <0.05, and the zones were classified according to Tukey's honestly significant difference (HSD) test. Regression analysis was performed by SigmaPlot 13.0 (Systat Software Inc., San Jose, CA, United States). Correlation coefficient between variables were calculated in by Pearson's correlation analysis, and p-values were acquired to present the significances of the linear fittings.

Winemaking Procedures

Vinification was conducted in 2016 and 2017 at the UC Davis Teaching and Research Winery. The grapes were harvested when Zone 1 reached a TSS of 29.88°Bx, 3.92 pH, 5.40 g·L^{-1} TA in 2016 and 26.72°Bx, 3.65 pH, 6.53 g·L^{-1} TA in 2017, and Zone 2 reached a TSS of 26.32°Bx, 3.75 pH, 6.01 g·L^{-1} TA in 2016 and 23.71°Bx, 3.58 pH, 7.22 g·L^{-1} TA in 2017. Before dividing the fruits from each zone into three dependent replicate fermentation vessels (200 L each), the grapes were destemmed and crushed once transported into the winery. 50 mg·L^{-1} of SO_2 was added to each vessel to prevent oxidation. Water was added to the musts to balance soluble solid level at 25°Bx due to the highly possible stuck fermentation events may occur based on the high TSS levels. Dilution factors were considered when analyzing the final wine chemical composition. The must samples were inoculated with EC-1118 yeast (Lallemand Lalvin®, Montreal, Canada) to initiate the fermentation in jacketed stainless steel tanks controlled by an integrated fermentation control system (T.J fermenters, Cypress Semiconductor Co., San Jose, CA, United States), and two volumes of must were pumped over twice per day by the system. The fermentations were carried at 25°C until the residual sugar contents were below 3 g·L^{-1}. Malolactic fermentation was initiated with the addition of Viniflora® *Oenococcus oeni* (Chr. Hansen A/S, Hørsholm, Denmark) at 12°C and 60% humidity. The free SO_2 levels were adjusted to 30 mg·L^{-1} after malolactic fermentation completed. Then the wines were sterile filtered and bottled before further chemical analysis. Wine samples were filtered by PTFE membrane filters (diameter: 13 mm, pore size: 0.45 μm, VWR, Seattle, WA, United States) and transferred directly into HPLC vials for anthocyanin analysis.

RESULTS

Weather at the Research Site

Between the 2 years of the study, the precipitation amounts were different (**Figure 1A**). The precipitation amount in the dormant season prior to 2016 was 559.5 mm (from previous harvest date to May as we reported previously; Brillante et al., 2017). However, this amount was 898 mm in the 2016–2017 season. The precipitation during growing seasons in these 2 years were limited, there were only 51.6 mm of precipitation received in 2016 from April to harvest. In 2017, 107 mm of precipitation were received from April to harvest. The research site only received 11.1 mm in 2016 and 15.4 mm in 2017 during the study time in each year from June to harvest. There was a slight difference observed close to harvest (**Figure 1B**). In 2016, GDD accumulation was 1183°C at harvest (5 October 2016). The GDD accumulation was greater in 2017 at 1220°C by harvest (20 September 2017). The cumulative ET_o was greater in 2017 compared to 2016 (**Figure 1C**). At harvest, the cumulative ET_o was 750 mm in 2016, but it was relatively lower compared to 872.8 mm in 2017.

Soil Property Assessment

Soil texture was measured at two different depths (**Figure 2**). In deep soil, the majority of the westerly section of the vineyard consisted mostly of loam with a small portion of clay loam in the southwestern corner of the vineyard, with the remainder being characterized as sandy clay loam (**Figure 2A**). In shallow soil, the easterly section of the vineyard mainly was a sandy clay loam with loam comprising the rest of shallow soil of the vineyard (**Figure 2B**).

Soil bulk EC was also assessed at two different depths by proximal sensing in the first season (**Figure 3**). In deep soil, EC values were lower in the majority of the westerly section of the vineyard (**Figure 3A**). In shallow soil, EC values were lower in the northwestern corner of the vineyard, and a small portion of the central section also showed lower EC values (**Figure 3B**).

Plant Water Status and Leaf Gas Exchange

Ψ_{stem} was continuously measured as previously reported in 2016 (Brillante et al., 2017) and 2017. Based on the interpolation of Ψ_{stem}, the trends in the calculated long-term Ψ_{stem} integral maps were similar to the trends in the soil bulk EC maps, especially when compared to the deep EC map (**Figure 4**). Majority of the westerly section of the vineyard had more water stress in 2016 (Brillante et al., 2017) as well as in 2017 (**Figure 4A**). Then, the interpolation maps of the Ψ_{stem} were separated into two zones by *k*-means clustering analysis as Year 1 was reported previously (Brillante et al., 2017). When comparing the two *k*-means clustering maps between 2016 and 2017, there was an 85% similarity according to Pearson's correlation coefficient between the two maps (**Figure 4B**). In 2017, the clustering map was 70 and 78% similar to the deep soil and shallow soil texture maps.

In 2017, Ψ_{stem} were consistently different between the two zones (**Figure 5A**), where Zone 2 consistently had higher Ψ_{stem} than Zone 1. Ψ_{stem} values became more negative with the progression of time, and the differences in Ψ_{stem} intensified throughout each season as berries reached a more advanced maturity. The differences between two zones ranged from 0.11 MPa on the first measurement day of 27 June to 0.31 MPa on the harvest day of 20 September. Between the two zones, a 0.22 MPa differences in Ψ_{stem} integrals were observed in 2017, similar to 0.21 MPa as in 2016 (Brillante et al., 2017).

Leaf gas exchange was measured 2017, where both years showed evident differences between the zones in both A_n and g_s (**Figure 5**). In 2017, the two zones showed significant differences in A_n and g_s with the highest values observed on 24 August (**Figures 5B,C**). Conversely, there was no consistent difference in WUE_i between the two zones in 2017, except Zone 2 showed higher WUEi on 13 July and lower WUEi on 24 August (**Figure 5D**).

Yield Components, Must Soluble Solids, pH, and Titratable Acidity

Components of yield were measured at harvest (**Table 1**, the harvest data on 5 October 2016 was reported previously in

Brillante et al., 2017), and berry primary metabolites were continuously assessed during 2016 and 2017 (**Figures 6**, **7**, the harvest data on 5 October 2016 was reported previously in Brillante et al., 2017). Between the two plant water status zones, there was no differences in yield, berry number, pruning weight, or Ravaz Index. However, there was an effect of experimental year where we measured greater yield and lower pruning weight per vine in Year 2. The only difference observed in yield components was that the berry skin weights were greater in Zone 1 in 2017.

The berry primary metabolites were different between the two zones in both years of the study. In 2016, berry weights were greater in Zone 2 on 28 July and 5 October when the fruits were harvested (**Figure 6A**). Zone 1 showed higher berry weights on 15 September. When the irrigation was stopped at veraison, TSS were higher in Zone 1 compared to Zone 2, which was measured on 1 September, 15 September, and 5 October (**Figure 6B**). At harvest, the fruits in Zone 1 reached a TSS of 29.9°Bx, while the ones in Zone 2 reached 26.3°Bx. The juice pH showed a similar result with TSS, where Zone 1 had higher pH in the last 3 months before harvest, except there was no difference shown on 15 September (**Figure 6C**). Berry TA was consistently higher in Zone 2 on all measured dates except 28 July (**Figure 6D**). At harvest, Zone 2 had 6.0 $g{\cdot}L^{-1}$ of TA, Zone 1 had 5.4 $g{\cdot}L^{-1}$.

In 2017, the differences in TSS, pH and TA were similar to 2016. Berry weights were higher in Zone 2 on 13 July and at harvest on 20 September (**Figure 7A**). The TSS increased more rapidly in Zone 1 close to harvest on 7 September and 20 September (**Figure 7B**) when compared to Zone 2. At harvest, TSS values were slightly lower than 2016 due to an earlier harvest time, where Zone 1 reached a TSS of 26.7°Bx, while Zone 2 reached 23.7°Bx (**Table 1**). The juice pH was higher in Zone 1 than Zone 2 at harvest as well (**Figure 7C**). Similar to 2016, the TA in the two zones was consistently different where Zone 2 had higher TA than Zone 1 on starting on 24 August until harvest (**Figure 7D**). At harvest, Zone 2 had 7.2 $g{\cdot}L^{-1}$ of TA, Zone 1 had 6.5 $g{\cdot}L^{-1}$.

Berry Skin Anthocyanins at Harvest

Berry skin anthocyanins were different between the two zones in 2016 (the harvest data on 5 October 2016 was partially reported previously in Brillante et al., 2017). Total delphinidins, petunidins, malvidins, and the sum of them as tri-hydroxylated anthocyanins were all higher in Zone 2 than Zone 1 (**Figures 8A,C,E,F,H**). Total cyanidins, peonidins, and the sum of them as di-hydroxylated anthocyanins were greater in Zone 2 on 23 August, 15 September, and at harvest (**Figures 8B,D,G**). Total skin anthocyanins were 2.2 mg per g of berry fresh weight (FW) in Zone 2 which was higher than the 1.85 mg measured in Zone 1 (**Table 2**).

In 2017, there were no differences between the two zones in delphinidin, cyanidin, petunidin, or peonidin at harvest (**Figures 9A–D**). Zone 1 had higher malvidins from 24 August until harvest, and tri-hydroxylated anthocyanins, total anthocyanins from 7 September until harvest (**Figures 9E,F,H**). Conversely, total malvidins, tri-hydroxylated anthocyanins, and total anthocyanins were higher in Zone 1 at harvest (**Table 2**). In Zone 2, we measured higher cyanidins and di-hydroxylated

TABLE 2 | Grape skin anthocyanins of Cabernet Sauvignon as separated by water status zoning in Sonoma County, CA in 2016 and 2017[a,b].

		Delphinidin ($mg{\cdot}g^{-1}$)	Cyanidin ($mg{\cdot}g^{-1}$)	Petunidin ($mg{\cdot}g^{-1}$)	Peonidin ($mg{\cdot}g^{-1}$)	Malvidin ($mg{\cdot}g^{-1}$)	Tri-OH ($mg{\cdot}g^{-1}$) [c]	Di-OH ($mg{\cdot}g^{-1}$)	Total anthocyanins ($mg{\cdot}g^{-1}$)
2016	Zone 1 ± SE[d]	0.34 ± 0.03	0.03 ± 0.01 b [e]	0.20 ± 0.01 b	0.15 ± 0.01 b	1.13 ± 0.05 b	1.67 ± 0.09 b	0.18 ± 0.02 b	1.85 ± 0.10 b
	Zone 2 ± SE	0.42 ± 0.03	0.06 ± 0.01 a	0.24 ± 0.01 a	0.20 ± 0.01 a	1.33 ± 0.06 a	1.99 ± 0.10 a	0.25 ± 0.02 a	2.24 ± 0.11 a
	p-value	ns	0.010	0.039	0.007	0.018	0.027	0.007	0.016
2017	Zone 1 ± SE	0.34 ± 0.01	0.05 ± 0.00	0.24 ± 0.01	0.17 ± 0.01	1.33 ± 0.04 a	1.91 ± 0.05 a	0.21 ± 0.01	2.12 ± 0.06 a
	Zone 2 ± SE	0.33 ± 0.02	0.05 ± 0.01	0.23 ± 0.01	0.18 ± 0.01	1.14 ± 0.02 b	1.70 ± 0.05 b	0.24 ± 0.02	1.94 ± 0.06 b
	p-value	ns	ns	0.002	ns	0.000	0.005	ns	0.000
Year		0.018	<0.0001	0.001	<0.0001	ns	ns	0.0001	ns
Year × Zoning		ns	ns	ns	ns	0.012	0.021	ns	0.020

[a]n = 35, ns: not significant, the data for 2016 in this table was partially published in Brillante et al. (2017), courtesy of American Chemical Society. [b]All compounds are expressed in the unit of mg per g of berry fresh weight. All anthocyanins comprised of their -glucoside, -acetyl glucoside, and -coumaroyl glucoside derivatives. [c]-OH, hydroxylation. Tri-OH included delphinidin, petunidin and malvidin, di-OH included cyanidin and peonidin. [d]Zone 1: lower plant water status zone, Zone 2: higher plant water status zone. Numbers in the column were expressed as their means ± standard error of the mean. [e]Different letters indicate significant mean separation according to Tukey's HSD test ($p < 0.05$).

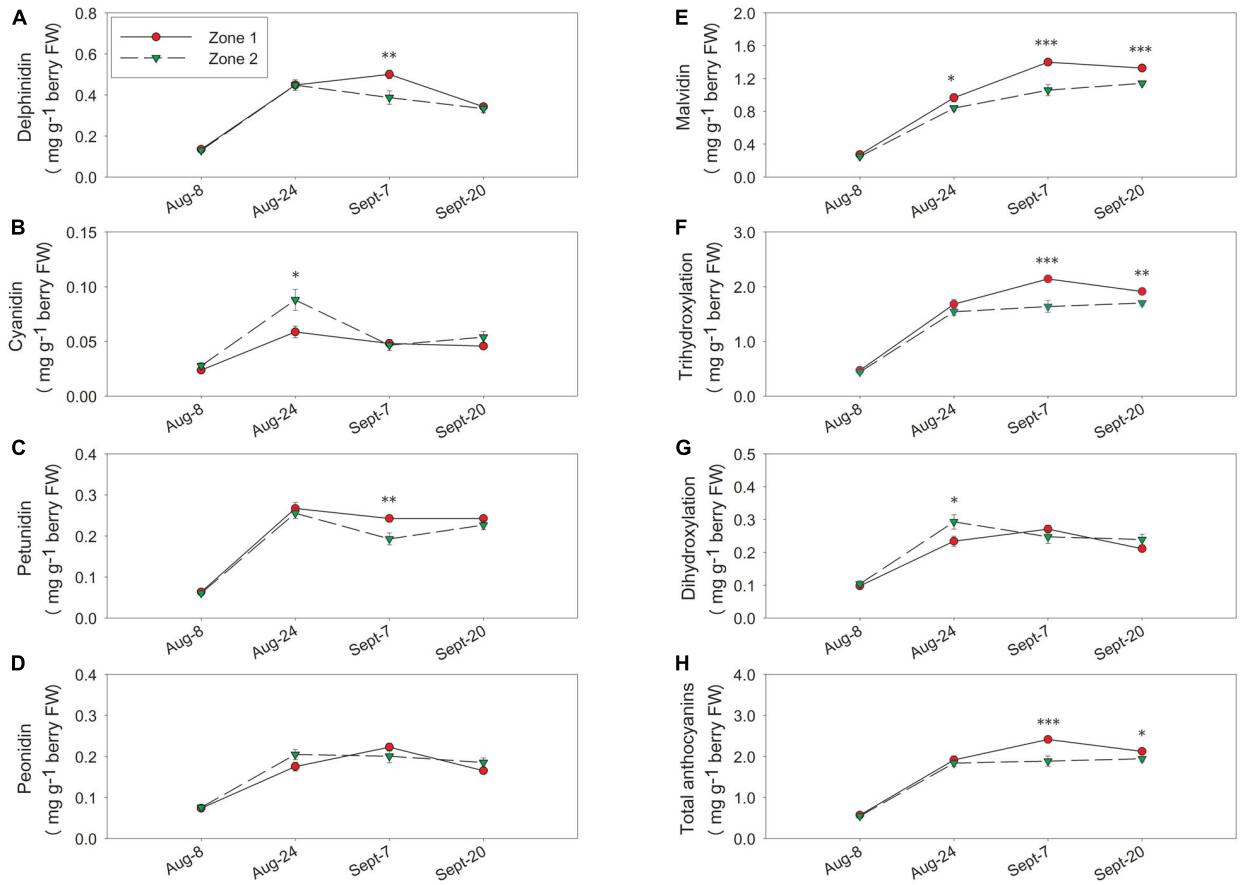

FIGURE 9 | Temporal development of grape berry anthocyanins between the two water status zones in 2017. **(A)** Delphinidins, **(B)** cyanidins, **(C)** petunidins, **(D)** peonidins, **(E)** malvidins, **(F)** tri-hydroxylation, **(G)** di-hydroxylation, **(H)** total anthocyanins. Error bars represent standard error of the mean. Asterisks represents significant levels p: ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$.

anthocyanins on 24 August (**Figures 9B,G**), and that was the only date Zone 2 had higher concentrations in any of these derivatives.

The temporal relationships between TSS and berry skin anthocyanins were investigated in both years (**Figure 10**). In both years, skin anthocyanins increased with the accumulation of TSS at first. In 2016, berry anthocyanins of Zone 1 had a significant decline in skin anthocyanins after 25°Bx TSS, resulting a lower concentration when compared to Zone 2 (**Figure 10A**). Conversely, the second season consistently showed greater anthocyanin concentration in Zone 1 than Zone 2 (**Figure 10B**). However, Zone 1 showed a more rapid decline after around 25°Bx TSS, and the skin anthocyanins were similar in values with Zone 2.

Wine Flavonoids

Wine-free anthocyanins and proanthocyanidins were assessed in both years. For anthocyanins, Zone 2 had higher concentrations of all derivatives in 2016, including tri-, di- hydroxylated, and total anthocyanins (**Table 3**). All the compounds were more than two times greater than Zone 1. However, there was no difference observed in any of these compounds in 2017. The overall concentrations of all these compounds were greater in 2017 than 2016.

For proanthocyanidins, similar results were observed (**Table 4**). In 2016, all the extension and terminal subunits were higher in Zone 2 than Zone 1. The amount of total proanthocyanidins were also higher in Zone 2. In 2017, however, there was no difference observed in any of these subunits or total proanthocyanidins. Again, the second season showed greater concentrations in all of these compounds compared to the first season. Neither year showed difference in mDP between the two zones.

Linking Soil to Grapevine Physiology

The relationships between soil bulk EC and whole grapevine physiology were investigated (**Table 5**). Soil bulk EC values at both depths increased when Ψ_{stem} became more positive, and soil bulk EC and Ψ_{stem} were significantly correlated in both seasons. The relationships between soil bulk EC and TSS reflected the relationships between soil bulk EC and Ψ_{stem}. They showed significant relations with each other in both years. In 2016, Ψ_{stem} showed a positive relationship with berry weight at harvest. No significant correlation was observed between soil bulk EC and

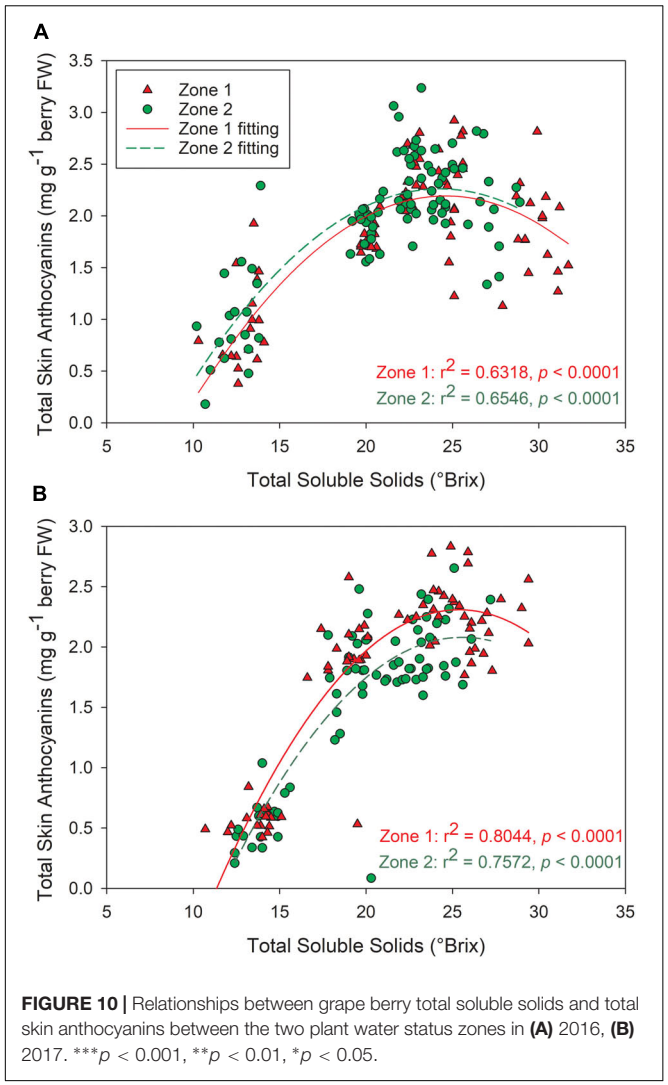

FIGURE 10 | Relationships between grape berry total soluble solids and total skin anthocyanins between the two plant water status zones in **(A)** 2016, **(B)** 2017. ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$.

berry weight. However, shallow soil bulk EC showed a positive correlation with berry weight besides Ψ_{stem} in 2017. Berry skin weight and total anthocyanins did not have any significant relationships with neither Ψ_{stem} nor soil bulk EC in 2017. In the same year, both berry skin weight and total anthocyanins were positively correlated with Ψ_{stem}, deep EC, and shallow EC. No parameters related to final yield except deep EC had a positive relationship with it in 2017.

DISCUSSION

Soil Bulk EC and Plant Water Status Spatial Relationships

Site topography influences plant water status (Brillante et al., 2017). In our previous work, we reported that absolute elevation of a vineyard was directly related to Y_{stem}. The correlation between Y_{stem} and elevation was significant and negative, indicating that the Y_{stem} would be lower when the elevation was higher. When soil moisture was model as wetness index,

TABLE 3 | Free anthocyanins in Cabernet Sauvignon wines as separated by water status zoning in Sonoma County, CA in 2016 and 2017[a,b].

		Delphinidin (mg·L^{-1})	Cyanidin (mg·L^{-1})	Petunidin (mg·L^{-1})	Peonidin (mg·L^{-1})	Malvidin (mg·L^{-1})	Tri-OH (mg·L^{-1})[c]	Di-OH (mg·L^{-1})	Total anthocyanins (mg·L^{-1})
2016	Zone 1 ± SE[d]	21.36 ± 0.36 b[e]	0.80 ± 0.01 b	21.01 ± 0.36 b	8.58 ± 0.13 b	163.69 ± 2.76 b	206.06 ± 3.45 b	9.38 ± 0.14 b	215.44 ± 3.59 b
	Zone 2 ± SE	51.35 ± 1.98 a	2.37 ± 0.09 a	45.45 ± 1.54 a	21.33 ± 0.75 a	350.23 ± 9.73 a	447.03 ± 13.25 a	27.53 ± 0.97 a	470.73 ± 14.08 a
	p-value	<0.0001	< 0.0001	<0.0001	< 0.0001	<0.0001	< 0.0001	<0.0001	< 0.0001
2017	Zone 1 ± SE	75.88 ± 1.46	4.94 ± 0.08	53.97 ± 0.92	33.42 ± 0.47	614.15 ± 8.48	743.99 ± 10.01	38.37 ± 0.54	782.36 ± 10.39
	Zone 2 ± SE	76.77 ± 1.65	4.82 ± 0.12	53.94 ± 0.99	33.40 ± 0.58	597.09 ± 9.75	727.80 ± 11.77	38.22 ± 0.69	766.02 ± 12.28
	p-value	Ns	ns	0.002	ns	0.000	0.005	ns	ns
Year		<0.0001	< 0.0001	<0.0001	< 0.0001	<0.0001	< 0.0001	<0.0001	< 0.0001
Year × Zoning		<0.0001	< 0.0001	<0.0001	< 0.0001	<0.0001	< 0.0001	<0.0001	< 0.0001

[a]n = 9, ns: not significant. [b]All compounds are expressed in the unit of mg per L. All anthocyanins comprised of their -glucoside, -acetyl glucoside, and -coumaroyl glucoside derivatives. [c] -OH, hydroxylation. Tri-OH included delphinidin, petunidin and malvidin, di-OH included cyanidin and peonidin. [d]Zone 1: lower plant water status zone, Zone 2: higher plant water status zone. Numbers in the column were expressed as their means ± standard error of the mean. [e]Different letters indicate significant mean separation according to Tukey's HSD test ($p < 0.05$).

TABLE 4 | Wine proanthocyanidin subunits of Cabernet Sauvignon as separated by water status zoning in Sonoma County, CA in 2016 and 2017[a,b].

		Extension subunits (mg·L^{-1})				Terminal subunits (mg·L^{-1})		Total proanthocyanins (mg·L^{-1})	mDP [c]
		EGC [c]	C [c]	EC [c]	ECG [c]	C [c]	EC [c]		
2016	Zone 1 ± SE [d]	75.07 ± 7.08 b[e]	10.07 ± 0.36 b	123.05 ± 9.13 b	2.73 ± 0.16 b	53.21 ± 1.57 b	21.39 ± 0.86 b	285.52 ± 18.14 b	3.73 ± 0.12
	Zone 2 ± SE	107.94 ± 10.05 a	14.14 ± 0.90 a	174.11 ± 12.40 a	3.70 ± 0.10 a	78.21 ± 1.97 a	38.75 ± 0.90 a	416.85 ± 22.70 a	3.51 ± 0.14
	p-value	0.017	0.001	0.004	<0.0001	< 0.0001	<0.0001	< 0.0001	ns
2017	Zone 1 ± SE	453.89 ± 40.68	33.83 ± 2.07	611.69 ± 46.57	9.18 ± 0.78	113.95 ± 9.33	106.00 ± 9.25	1328.55 ± 86.78	5.90 ± 0.38
	Zone 2 ± SE	519.56 ± 68.02	38.77 ± 4.42	690.87 ± 80.86	14.21 ± 2.67	139.08 ± 18.43	127.54 ± 6.85	1530.03 ± 169.24	5.59 ± 0.41
	p-value	Ns	ns	ns	ns	ns	ns	ns	ns
Year		<0.0001	< 0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	< 0.0001
Year × Zoning		Ns	ns	ns	0.012	ns	ns	ns	ns

[a] *n = 9, ns: not significant.* [b] *All compounds are expressed in the unit of mg per L.* [c] *C, (+)-catechin; EC, (-)-epicatechin; ECG, (-)-epicatechin-3-O-gallate; EGC, (-)-epigallocatechin; mDP: mean degree of polymerization. mDP was calculated as the ratio of total proanthocyanidins to the terminal subunits.* [d] *Zone 1: lower plant water status zone, Zone 2: higher plant water status zone. Numbers in the column were expressed as their means ± standard error of the mean.* [e] *Different letters indicate significant mean separation according to Tukey's HSD test ($p < 0.05$).*

it indicated a negative and significant relationship with Y_{stem} but the relationship was not linear. In our previous work, we were unable to deduce a significant relationship between site topography variables such as absolute elevation and berry chemistry (Brillante et al., 2017). Bramley et al. (2011a) showed that soil bulk EC was directly related to soil clay content, which was contradictory to our findings. We attributed this discrepancy to the relatively stable soil texture throughout the season or even several seasons. On the other hand, the effect of soil water content might be the major factor to influence plant development during the season. The soil texture and soil bulk EC sensing analysis conducted in this study were able to explain the variability in plant water status that the site topography could not. Soil texture and soil bulk EC can be related to spatial differences in soil water availability (Tramontini et al., 2013). Specifically, soil texture is a determinant of soil water holding capacity, hence affecting the amount of water available to the plants. In our study, the western section of the vineyard had greater loam proportion, where the grapevines were experiencing more severe water deficits (Brillante et al., 2017). The eastern section had more sandy soil in both deep and shallow soil, where the grapevines were under less severe water deficits. Our findings are corroborated with previous work, where clay soil would lead to less plant available water, although clay soil had higher water holding capacity than sandy soil (Tramontini et al., 2013). Furthermore, Cabernet Sauvignon grapevines grown in clay soil would result in lower g_s and A_n compared to grapevines grown in soils that had higher proportion of sandy soils (Yu and Kurtural, 2020).

There was evident variability in soil bulk EC in this study. Previous studies reported that when soil bulk EC was proximally sensed, it was closely related to soil water content (Bittelli, 2011; Brillante et al., 2015). We found that soil bulk EC was consistently and directly related to long-term Ψ_{stem} over the course of our study. Our findings are corroborated by previous works (Rodríguez-Pérez et al., 2011; Brillante et al., 2014), where higher soil bulk EC values corresponded to higher soil water content. Previous studies suggested that the relationship between soil water content and soil bulk EC was soil-specific, and needed to include soil chemical and physical properties to explain variability and plant water status (Morari et al., 2009; Brillante et al., 2016b). Due to the limited amount of water put into wine grape vineyards, soil water content would be the major factor affecting soil electrical properties rather than the residual salinity after water evaporation from soil. The significant relationship between soil bulk EC and Ψ_{stem} in this study agreed with previous studies, indicating the possibility of soil bulk EC sensing being used to assess plant water status (Bramley et al., 2011a; Rodríguez-Pérez et al., 2011). Moreover, in our study, the spatial variability in grapevine physiology reflected the variability in soil bulk EC very well when assessed by proximal sensing. Due to the relationship of soil bulk EC on the amount of available water to plants reported in previous research (Rodríguez-Pérez et al., 2011; Brillante et al., 2014), this approach had been utilized to identify the variability in the plant physiology based on the soil sensing technologies and apply targeted management strategies (Bramley et al., 2011a), and our study provided more evidence toward the feasibility of it.

TABLE 5 | Correlation matrices, values were expressed in Pearson Correlation values of "r" in a commercial Cabernet Sauvignon vineyard in Sonoma County, CA in 2016 and 2017[a,b].

		SWP Int	Deep EC	Shallow EC	TSS	Berry weight	Skin weight	TSA	Yield
2016	SWP Int		0.6***[c]	0.68***	−0.81***	0.46**	0.22	0.24	0.19
	Deep EC	0.6***		0.5**	−0.69***	0.03	0.07	0.24	0.17
	Shallow EC	0.68***	0.5**		−0.57***	0.25	0.22	0.27	0.06
2017	SWP Int		0.73***	0.59***	−0.83***	0.49**	0.49**	0.6***	0.20
	Deep EC	0.73***		0.5**	−0.68***	0.18	0.35*	0.53**	0.41*
	Shallow EC	0.59***	0.5**		−0.67***	0.51**	0.55**	0.39*	0.02

*[a]df = 33. [b]SWP Int: stem water potential integrals, EC: soil bulk electrical conductivity, TSS: total soluble solids, TSA: total skin anthocyanins. [c]Asterisks represents significant levels p: ***p < 0.001, **p < 0.01, *p < 0.05.*

The variability we measured proximally in soil characteristics was reflected in plant water status and leaf gas exchange in our study. Previous research had reported that variable soil characteristics in space would cause spatial variations in plant water status (Brillante et al., 2016a). Although the precipitation amounts were vastly different between the two dormant seasons, the uniformly scheduled irrigation did not ameliorate the natural spatial variability in plant water status induced by soil properties. On the contrary, the separations in plant water status and leaf gas exchange were already significant even before the irrigation ceased after veraison. This proved that the spatial variability in the soil dominated the accessibility of the available soil water toward the plant, and made the spatial variability expressed in the grapevine. Our results in the second year corroborated those of the first year, showing that the separation in both plant water status and leaf gas exchange between the two zones were consistent.

Leaf gas exchange was closely related to plant water status, and this relationship was shown in previous research (Costa et al., 2012). The relationships between leaf gas exchange and plant water status were evident in our study, where a higher Ψ_{stem} would promote a greater stomatal conductance to increase carbon assimilation capacity and decrease intrinsic water use efficiency. In our study, the lowest Ψ_{stem} we observed were around harvest with Ψ_{stem} of -1.6 MPa and g_s of around 50 mmol H_2O $m^{-2} \cdot s^{-1}$, which were not severe enough to impair berry ripening although the photosynthetic activities were still affected. Overall, the g_s and A_N reached the maximum values at veraison and declined with decreasing plant water status and leaf age toward the end of the season. This further affirmed that the continuous water deficits during the growing season, especially being more pronounced after irrigation was ended after veraison, would reduce stomatal conductance. The water deficits would act as passive hydraulic signals or active hormonal signals with the upregulation in abscisic acid (ABA) synthesis to limit plant photosynthetic activities, hence lower g_s and A_N values (Costa et al., 2012; Tombesi et al., 2015).

Components of Yield

According to the previous research, components of yield may be affected by plant water status, where higher water deficits would result in reductions of yield, berry skin weight, and berry weight (Williams, 2010; Korkutal et al., 2011; Santesteban et al., 2011). In our study, we observed constant separation in plant water status after veraison. However, there was no difference shown in cluster number, yield, berry number, or pruning weight. The only difference measured in yield components was that berry skin weight was higher in Zone 1 in the second season. Early season water deficit irrigation (prior to veraison) had higher probability to decrease yield than later season water deficit irrigation (post-veraison to harvest). However, a season-long water deficit irrigation would have the lowest yield even despite the season-long water deficit irrigation regime applying double amount of water than the other regimes (Tarara et al., 2011). Some other studies did not have the same results, as early water deficit irrigation did not show significant influences on yield compared to late water deficit irrigation (Intrigliolo and Castel, 2010; Intrigliolo et al., 2012). Another possible explanation was that Zone 1 had greater water amount held in the soil due to the higher clay content. The clay soil with higher water-holding capacity had a better water status at the early season compared to Zone 2, even though the sandy soil in Zone 2 would benefit the plant growth with irrigation when the season progressed (Tramontini et al., 2013). The later season water deficit was exacerbated in Zone 1 due to its higher clay content, causing Zone 1 lost the benefits from the high water status in the early season, and eventually had similar yield components with Zone 2 at harvest. In our work, we did not see any evidence of Ravaz index being affected by spatial variability of plant water status. These results were corroborated by Terry and Kurtural when grapevine cultivar 'Syrah' was exposed to post-veraison water deficits in comparable severity of -1.4 MPa (Terry and Kurtural, 2011).

Must-Soluble Solids, pH, and Titratable Acidity

Water deficits affect advancement of grape berry maturity, they promote TSS accumulation and TA degradation in grape berries (Basile et al., 2011; Williams, 2012). Two factors contributed to these differences between the two zones. First, a greater water deficit advanced the berry maturation, leading to a higher TSS and lower TA (Escalona et al., 2015). Second, berry dehydration may have occurred and the TSS concentration increased in the berries. In our study, smaller berries were observed in Zone 1, which can confirm the berry dehydration could have led to higher TSS in Zone 1. As for berry TA, one study showed that grape organic acids biodegradation would be faster with more solar radiation and higher temperature (Cholet et al., 2016). Although the acid degradation was not related to water deficits, like mentioned above, water deficits would limit the grapevines' ability to regulate temperature (Tombesi et al., 2015). Thus, water

deficits could promote the organic acid degradation and this effect was observed in this study.

Berry Skin and Wine Flavonoids

Mild water deficits increased the flavonoid content and concentration of red-skinned grape berry due to the upregulation in flavonoid synthesis and the advancement of berry dehydration during growing season (Castellarin et al., 2007a; Bondada and Shutthanandan, 2012). A positive relationship was noticed between soil bulk EC and total skin anthocyanins in 2017 at both depths of soil bulk EC measurements. A more prolonged severe water deficit would lead to deleterious stomatal and temperature regulation and eventually resulted in flavonoid degradation, specifically anthocyanins (Movahed et al., 2016). This was a plausible explanation for the non-significant relationship between soil bulk EC and total skin anthocyanins in 2016, wherein harvest took place at higher soluble solids and Zone 1 berry skin anthocyanins were presumably in decline. Furthermore, the berry weights were higher in Zone 2, which was similar to the observations in our previous work (Martínez-Lüscher et al., 2017), indicating there was less berry dehydration. Thus, the higher anthocyanins in Zone 2 was mainly due to the upregulation in anthocyanins other than anthocyanins degradation. These effects were also observed in the wines of 2016, where Zone 2 had higher anthocyanin concentrations. However, in the second season, the differences in berry skin anthocyanins at harvest did not carry over into the wines. We contributed this to the more advanced berry maturity levels at harvest in the first season, the skin cell walls could have become more porous during ripening and increased the extractability of flavonoid compounds (Bindon et al., 2014). With relatively greater amounts of flavonoids extracted, there was a higher chance to pass on the separations of anthocyanins from the berries to the wines.

Grape berry skin proanthocyanidins are less sensitive toward water deficits than anthocyanins (Castellarin et al., 2007a; Cáceres-Mella et al., 2017). Nevertheless, their biosynthesis and concentration may be modified by water deficits (Ollé et al., 2011; Cáceres-Mella et al., 2017). In 2016, wine total proanthocyanidins and all the subunits were greater in Zone 2. These differences were not observed in the second season. We attributed this lack of consistency in proanthocyanidin disparities between the two zones to the more advanced maturity of the berries were harvested in 2016 than in 2017. We suggest that similar to skin anthocyanins, the more advanced berry maturity in 2016 could have promoted the proanthocyanidin extractability in the skin tissues (Bindon et al., 2014), which may augment the separations in the concentration of all the subunits between the two zones.

CONCLUSION

Our work provided evidence of the connection between soil bulk EC sensing and whole plant physiology, and the effects of which then cascaded to berry and wine chemistry. We presented that soil bulk EC in vineyard systems affected plant water status. The clusters of plants with similar water status may comprise zones of similar physiological behavior due to these inherent differences from different plant water status, and the discrepancies in plant water status resulted in cascading effects on berry chemistry. In conclusion, our work provides fundamental knowledge about the applicability of soil bulk EC sensing in the vineyards, and its potential directional utilization by connecting proximal sensing to spatial distribution of whole-plant physiological performance together with berry and wine chemistry.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

AUTHOR CONTRIBUTIONS

SK acquired the funding and designed the trial. LB, RY, and JM-L executed the trial. RY made the wine, analyzed the metabolites, and wrote the first version of the manuscript. All authors contributed to the final version and approved.

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REFERENCES

Basile, B., Marsal, J., Mata, M., Vallverdú, X., Bellvert, J., and Girona, J. (2011). Phenological sensitivity of Cabernet Sauvignon to water stress: vine physiology and berry composition. *Am. J. Enol. Viticult.* 62, 452–461. doi: 10.5344/ajev.2011.11003

Bindon, K. A., Madani, S. H., Pendleton, P., Smith, P. A., and Kennedy, J. A. (2014). Factors affecting skin tannin extractability in ripening grapes. *J. Agric. Food Chem.* 62, 1130–1141. doi: 10.1021/jf4050606

Bittelli, M. (2011). Measuring soil water content: a review. *HortTechnology* 21, 293–300. doi: 10.21273/horttech.21.3.293

Bondada, B., and Shutthanandan, J. (2012). Understanding differential responses of grapevine (*Vitis vinifera* L.) leaf and fruit to water stress and recovery following re-watering. *Am. J. Plant Sci.* 3:1232. doi: 10.4236/ajps.2012.39149

Bonfante, A., Agrillo, A., Albrizio, R., Basile, A., Buonomo, R., De Mascellis, R., et al. (2015). Functional homogeneous zones (fHZs) in viticultural zoning procedure: an Italian case study on Aglianico vine. *Soil* 1, 427–441. doi: 10.5194/soil-1-427-2015

Bramley, R., Ouzman, J., and Boss, P. (2011a). Variation in vine vigour, grape yield and vineyard soils and topography as indicators of variation in the chemical composition of grapes, wine and wine sensory attributes. *Aust. J. Grape Wine Res.* 17, 217–229. doi: 10.1111/j.1755-0238.2011.00136.x

Bramley, R., Ouzman, J., and Thornton, C. (2011b). Selective harvesting is a feasible and profitable strategy even when grape and wine production is geared towards large fermentation volumes. *Aust. J. Grape Wine Res.* 17, 298–305. doi: 10.1111/j.1755-0238.2011.00151.x

Bramley, R., Trought, M. C., and Praat, J. P. (2011c). Vineyard variability in Marlborough, New Zealand: characterising variation in vineyard performance and options for the implementation of Precision Viticulture. *Aust. J. Grape Wine Res.* 17, 72–78. doi: 10.1111/j.1755-0238.2010.00119.x

Brillante, L., Bois, B., Lévêque, J., and Mathieu, O. (2016a). Variations in soil-water use by grapevine according to plant water status and soil physical-chemical characteristics—A 3D spatio-temporal analysis. *Eur. J. Agron.* 77, 122–135. doi: 10.1016/j.eja.2016.04.004

Brillante, L., Bois, B., Mathieu, O., and Lévêque, J. (2016b). Electrical imaging of soil water availability to grapevine: a benchmark experiment of several machine-learning techniques. *Precision Agricult.* 17, 637–658. doi: 10.1007/s11119-016-9441-1

Brillante, L., Bois, B., Mathieu, O., Bichet, V., Michot, D., and Lévêque, J. (2014). Monitoring soil volume wetness in heterogeneous soils by electrical resistivity. A field-based pedotransfer function. *J. Hydrol.* 516, 56–66. doi: 10.1016/j.jhydrol.2014.01.052

Brillante, L., Martinez-Luscher, J., Yu, R., Plank, C. M., Sanchez, L., Bates, T. L., et al. (2017). Assessing spatial variability of grape skin flavonoids at the vineyard scale based on plant water status mapping. *J. Agric. Food Chem.* 65, 5255–5265. doi: 10.1021/acs.jafc.7b01749

Brillante, L., Mathieu, O., Bois, B., Van Leeuwen, C., and Lévêque, J. (2015). The use of soil electrical resistivity to monitor plant and soil water relationships in vineyards. *Soil* 1, 273–286. doi: 10.5194/soil-1-273-2015

Bucchetti, B., Matthews, M. A., Falginella, L., Peterlunger, E., and Castellarin, S. D. (2011). Effect of water deficit on Merlot grape tannins and anthocyanins across four seasons. *Sci. Horticult.* 128, 297–305. doi: 10.1016/j.scienta.2011.02.003

Cáceres-Mella, A., Talaverano, M. I., Villalobos-González, L., Ribalta-Pizarro, C., and Pastenes, C. (2017). Controlled water deficit during ripening affects proanthocyanidin synthesis, concentration and composition in Cabernet Sauvignon grape skins. *Plant Physiol. Biochem.* 117, 34–41. doi: 10.1016/j.plaphy.2017.05.015

Castellarin, S. D., Matthews, M. A., Di Gaspero, G., and Gambetta, G. A. (2007a). Water deficits accelerate ripening and induce changes in gene expression regulating flavonoid biosynthesis in grape berries. *Planta* 227, 101–112. doi: 10.1007/s00425-007-0598-8

Castellarin, S. D., Pfeiffer, A., Sivilotti, P., Degan, M., Peterlunger, E., and Di Gaspero, G. (2007b). Transcriptional regulation of anthocyanin biosynthesis in ripening fruits of grapevine under seasonal water deficit. *Plant Cell Environ.* 30, 1381–1399. doi: 10.1111/j.1365-3040.2007.01716.x

Charrad, M., Ghazzali, N., Boiteau, V., and Niknafs, A. (2014). NbClust: an R Package for determining the relevant number of clusters in a data set. *J. Stat. Softw.* 61:36.

Cholet, C., Claverol, S., Claisse, O., Rabot, A., Osowsky, A., Dumot, V., et al. (2016). Tartaric acid pathways in *Vitis vinifera* L.(cv. Ugni blanc): a comparative study of two vintages with contrasted climatic conditions. *BMC Plant Biol.* 16:144. doi: 10.1186/s12870-016-0833-1

Cortell, J. M., Halbleib, M., Gallagher, A. V., Righetti, T. L., and Kennedy, J. A. (2007). Influence of vine vigor on grape (*Vitis vinifera* L. cv. Pinot Noir) anthocyanins. 1. Anthocyanin concentration and composition in fruit. *J. Agricult. Food Chem.* 55, 6575–6584. doi: 10.1021/jf070195v

Costa, J. M., Ortuño, M. F., Lopes, C. M., and Chaves, M. M. (2012). Grapevine varieties exhibiting differences in stomatal response to water deficit. *Funct. Plant Biol.* 39, 179–189.

Dai, Z. W., Meddar, M., Renaud, C., Merlin, I., Hilbert, G., Delrot, S., et al. (2014). Long-term in vitro culture of grape berries and its application to assess the effects of sugar supply on anthocyanin accumulation. *J. Exp. Bot.* 65, 4665–4677. doi: 10.1093/jxb/ert489

Escalona, J., Bota, J., and Medrano, H. (2015). Distribution of leaf photosynthesis and transpiration within grapevine canopies under different drought conditions. *VITIS J. Grapevine Res.* 42:57.

Gonzalo-Diago, A., Dizy, M., and Fernaìndez-Zurbano, P. N. (2013). Taste and mouthfeel properties of red wines proanthocyanidins and their relation to the chemical composition. *J. Agricult. Food Chem.* 61, 8861–8870. doi: 10.1021/jf401041q

Hiemstra, P. (2013). *Package 'automap'. R Package*.

Hijmans, R. J., Van Etten, J., Cheng, J., Mattiuzzi, M., Sumner, M., Greenberg, J. A., et al. (2015). *Package 'raster'. R package*.

Intrigliolo, D., Pérez, D., Risco, D., Yeves, A., and Castel, J. (2012). Yield components and grape composition responses to seasonal water deficits in Tempranillo grapevines. *Irrigat. Sci.* 30, 339–349. doi: 10.1007/s00271-012-0354-0

Intrigliolo, D. S., and Castel, J. R. (2010). Response of grapevine cv.'Tempranillo'to timing and amount of irrigation: water relations, vine growth, yield and berry and wine composition. *Irrigati. Sci.* 28:113. doi: 10.1007/s00271-009-0164-1

Kennedy, J. A., and Jones, G. P. (2001). Analysis of proanthocyanidin cleavage products following acid-catalysis in the presence of excess phloroglucinol. *J. Agric. Food Chem.* 49, 1740–1746. doi: 10.1021/jf001030o

Korkutal, I., Bahar, E., and Carbonneau, A. (2011). Growth and yield responses of cv. Merlot (*Vitis vinifera* L.) to early water stress. *Afr. J. Agricult. Res.* 6, 6281–6288.

Lorrain, B., Ky, I., Pechamat, L., and Teissedre, P.-L. (2013). Evolution of analysis of polyhenols from grapes, wines, and extracts. *Molecules* 18, 1076–1100. doi: 10.3390/molecules18011076

Martínez-Lüscher, J., Brillante, L., and Kurtural, S. K. (2019). Flavonol profile is a reliable indicator to assess canopy architecture and the exposure of red wine grapes to solar radiation. *Front. Plant Sci.* 10:10. doi: 10.3389/fpls.2019.00010

Martínez-Lüscher, J., Brillante, L., Nelson, C. C., Al-Kereamy, A. M., Zhuang, S., and Kurtural, S. K. (2017). Precipitation before bud break and irrigation affect the response of grapevine 'Zinfandel'yields and berry skin phenolic composition to training systems. *Sci. Horticult.* 222, 153–161. doi: 10.1016/j.scienta.2017.05.011

Martínez-Lüscher, J., Sánchez-Díaz, M., Delrot, S., Aguirreolea, J., Pascual, I., and Gomès, E. (2014). Ultraviolet-B radiation and water deficit interact to alter flavonol and anthocyanin profiles in grapevine berries through transcriptomic regulation. *Plant Cell Physiol.* 55, 1925–1936. doi: 10.1093/pcp/pcu121

Matese, A., Toscano, P., Di Gennaro, S., Genesio, L., Vaccari, F., Primicerio, J., et al. (2015). Intercomparison of UAV, aircraft and satellite remote sensing platforms for precision viticulture. *Remote Sens.* 7, 2971–2990. doi: 10.3390/rs70302971

Morari, F., Castrignanò, A., and Pagliarin, C. (2009). Application of multivariate geostatistics in delineating management zones within a gravelly vineyard using geo-electrical sensors. *Comput. Electr. Agricult.* 68, 97–107. doi: 10.1016/j.compag.2009.05.003

Movahed, N., Pastore, C., Cellini, A., Allegro, G., Valentini, G., Zenoni, S., et al. (2016). The grapevine VviPrx31 peroxidase as a candidate gene involved in anthocyanin degradation in ripening berries under high temperature. *J. Plant Res.* 129, 513–526. doi: 10.1007/s10265-016-0786-3

Ollé, D., Guiraud, J.-L., Souquet, J. M., Terrier, N., Ageorges, A., Cheynier, V., et al. (2011). Effect of pre-and post-veraison water deficit on proanthocyanidin and anthocyanin accumulation during Shiraz berry development. *Aust. J. Grape Wine Res.* 17, 90–100. doi: 10.1111/j.1755-0238.2010.00121.x

Pebesma, E. J. (2004). Multivariable geostatistics in S: the gstat package. *Comput. Geosci.* 30, 683–691. doi: 10.1016/j.cageo.2004.03.012

Petrussa, E., Braidot, E., Zancani, M., Peresson, C., Bertolini, A., Patui, S., et al. (2013). Plant flavonoids—biosynthesis, transport and involvement in stress responses. *Int. J. Mol. Sci.* 14, 14950–14973. doi: 10.3390/ijms140714950

R Core Team, (2019). *R: A Language and Environment for Statistical Computing*. Vienna: R Core Team.

Rodríguez-Pérez, J. R., Plant, R. E., Lambert, J.-J., and Smart, D. R. (2011). Using apparent soil electrical conductivity (EC a) to characterize vineyard soils of high clay content. *Precis. Agricult.* 12, 775–794. doi: 10.1007/s11119-011-9220-y

Santesteban, L., Miranda, C., and Royo, J. (2011). Regulated deficit irrigation effects on growth, yield, grape quality and individual anthocyanin composition in *Vitis vinifera* L. cv.'Tempranillo'. *Agricult. Water Manag.* 98, 1171–1179. doi: 10.1016/j.agwat.2011.02.011

Scarlett, N., Bramley, R., and Siebert, T. (2014). Within-vineyard variation in the 'pepper'compound rotundone is spatially structured and related to variation in the land underlying the vineyard. *Aust. J. Grape Wine Res.* 20, 214–222. doi: 10.1111/ajgw.12075

Smart, R. E., and Coombe, B. G. (1983). *Water Relations of Grapevines [Vitis]. Water Deficits and Plant Growth*. Paris: FAO.

Spayd, S. E., Tarara, J. M., Mee, D. L., and Ferguson, J. (2002). Separation of sunlight and temperature effects on the composition of *Vitis vinifera* cv. Merlot berries. *Am. J. Enol. Viticult.* 53, 171–182.

Su, S. L., Singh, D., and Baghini, M. S. (2014). A critical review of soil moisture measurement. *Measurement* 54, 92–105. doi: 10.1016/j.measurement.2014.04.007

Tagarakis, A., Liakos, V., Fountas, S., Koundouras, S., and Gemtos, T. (2013). Management zones delineation using fuzzy clustering techniques in grapevines. *Precis. Agricult.* 14, 18–39. doi: 10.1007/s11119-012-9275-4

Tarara, J. M., Peña, J. E. P., Keller, M., Schreiner, R. P., and Smithyman, R. P. (2011). Net carbon exchange in grapevine canopies responds rapidly to timing and extent of regulated deficit irrigation. *Funct. Plant Biol.* 38, 386–400.

Terry, D. B., and Kurtural, S. K. (2011). Achieving vine balance of Syrah with mechanical canopy management and regulated deficit irrigation. *Am. J. Enol. Viticult.* 62, 426–437. doi: 10.5344/ajev.2011.11022

Tombesi, S., Nardini, A., Frioni, T., Soccolini, M., Zadra, C., Farinelli, D., et al. (2015). Stomatal closure is induced by hydraulic signals and maintained by ABA in drought-stressed grapevine. *Sci. Rep.* 5:12449.

Tramontini, S., Van Leeuwen, C., Domec, J.-C., Destrac-Irvine, A., Basteau, C., Vitali, M., et al. (2013). Impact of soil texture and water availability on the hydraulic control of plant and grape-berry development. *Plant Soil* 368, 215–230. doi: 10.1007/s11104-012-1507-x

Williams, L. E. (2010). Interaction of rootstock and applied water amounts at various fractions of estimated evapotranspiration (ETc) on productivity of Cabernet Sauvignon. *Aust. J. Grape Wine Res.* 16, 434–444. doi: 10.1111/j.1755-0238.2010.00104.x

Williams, L. E. (2012). Interaction of applied water amounts and leaf removal in the fruiting zone on grapevine water relations and productivity of Merlot. *Irrigat. Sci.* 30, 363–375. doi: 10.1007/s00271-012-0355-z

Yu, R., and Kurtural, S. K. (2020). Proximal sensing of soil electrical conductivity provides a link to soil-plant water relationships and supports the identification of plant water status zones in vineyards. *Front. Plant Sci.* 11:244. doi: 10.3389/fpls.2020.00244

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Sensitivity of Grapevine Soil–Water Balance to Rainfall Spatial Variability at Local Scale Level

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In rainfed agriculture systems, rainfall water management (harvesting, storage, and efficient use) is a key issue. At local scale (i.e., from 100 m to 50 km), the impact of rainfall spatial and temporal variability on crop water availability is seldom addressed. In order to accurately depict the space and time variations of rainfall at local scale, a dense rain-gauges network composed of 45 rain-gauges has been deployed over 28-km^2 area, in Burgundy vineyards (North-East France). Rainfall data collected by each rain gauge from 2014 to 2016 were used as input variables in the Lebon et al. (2003) grapevine water balance model. All other climate variables, vineyard, and soil parameters were kept the same for each simulation in order to capture the impact of the sole spatial variability of rainfall on vineyard water status. As rainfall dynamics impact on the vineyard depends on the soil water content, water balance was modeled considering soils with low (50 mm) and medium (150 mm) soil water-holding capacities, representative of the soils of the area. The impact of modeled soil water availability for grapevine was assessed using the water deficit stress index (WDSI), i.e., the relative stomatal conductance. Local rainfall variability throughout the vine vegetative period leads to large variations in WDSI; it varied up to 0.3 within the study area due to because of rainfall spatial variability. Using a set of 34 weather stations at mesoscale level over Burgundy (186 km from North to South), we showed that local rainfall might contribute to change in grapevine water status as large as 50% of the simulated regional water balance spatial variability. Our results indicate that local rainfall and its impacts on agricultural production are probably not sufficiently considered in farming systems, potentially leading to inaccurate water management (cover-crop, irrigation) due to sparse rainfall network.

Keywords: water balance, grapevine, rainfall, local scale, Burgundy, terroir

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INTRODUCTION

Water management throughout the 21st century is a widely documented and certainly a challenging matter (see for example Clothier et al., 2010). Growing population, changes in food quality, and development of non-feeding agricultural products (such as bioenergy) will undoubtedly lead to increased pressure on natural resources, including water. Crop water consumption is expected to

grow from 30 to 53% in 2050, in comparison the early 21st century (de Fraiture and Wichelns, 2010). Therefore, large improvements in both rainfed and irrigated agriculture are required to limit water demand and to provide sufficient food and limit the impacts of crop production on the environment. This can be achieved through adapted and improved agronomical practices as well as regulations imposed by regional and global policies (Howell, 2001; Ward and Michelsen, 2002; Fedoroff et al., 2010).

At the field scale, water can be saved by the use of high water-use efficiency plant material (Condon et al., 2004; Marguerit et al., 2012), soil management techniques (Hatfield et al., 2001), or accurate irrigation systems (Howell, 2001) and planning (Wang et al., 2001; Ali and Talukder, 2008).

For the specific case of rainfed agriculture, water management strongly relies on rainfall water harvesting, water storage, reducing non-productive evaporation, increasing plant water uptake capacity (e.g., with optimum crop geometry, conservation agriculture. . .), and increasing the water use efficiency of crops (through adapted plant species and varieties) (Rockström et al., 2010; Rossato et al., 2017).

To develop accurate strategies and policies to collect and save rainfall water, fine knowledge of space and time rainfall patterns is necessary. However, even at local scale [i.e., from 100 m to 50 km, according to Oke (1987)], the spatial variability of rainfall can lead to large variations in the spatial distribution of water resources (Finnerty et al., 1997), specifically during convective events (Duncan et al., 1993). Numerous studies have documented the substantial variability of rainfall at local scale (e.g., Berne et al., 2004; Ciach and Krajewski, 2006; Villarini et al., 2008) and its impact on potential rainfall erosivity (Fiener and Auerswald, 2009). Accounting for rainfall spatial variability has been previously suggested as it can be suspected to affect experimental trials results for agriculture (Sivakumar and Hatfield, 1990). We address in this paper the potential impact of rainfall local spatial variability on agriculture, using grapevine as a reference cropping system.

Grapes is a crop for which water management produced a significant body of scientific literature. Grapevine requires limited input of water. When it suffers a moderate water deficit during the fruit development, it produces grapes with high-quality potential for winemaking (Seguin, 1986; van Leeuwen et al., 2009; Acevedo-Opazo et al., 2010). Water status is often considered as a key factor of the so-called "Terroir effect," a concept that bounds the sensory characteristics of a product to its area of production, because of various factors among which soil and climate conditions (van Leeuwen, 2010). As for other crops, severe water deficit reduces yield (Hardie and Considine, 1976). The environmental and agronomical factors affecting grapevine water status are largely documented (Deloire et al., 2004; Vaudour et al., 2015). At local scale, soil water status variability is addressed mainly through soil, topography, and plant-based studies (André et al., 2012; Bellvert et al., 2013; Bonfante et al., 2015; Brillante et al., 2016a,b). The role of climate variation at local scale on grapevine water status has recently been considered through terrain impact on radiative balance (and therefore vineyard evapotranspiration) and rainfall runoff using water balance modeling (Hofmann et al., 2014).

Local scale weather variability has been increasingly studied lately in vineyards (Quénol, 2014), although mostly focusing on air temperature and its impact on grapevine precocity. Considerable variations in temperature have been recently reported (Quénol and Bonnardot, 2014), up to 300°days (Winkler index) within less than 2-km distance (Bonnefoy et al., 2013). Yet, impact of rainfall spatial variability at this scale on viticulture has not been addressed so far.

The current article explores the potential impact of local rainfall space and time variability on grapevine water availability through the response of a grapevine soil–water balance model to local rainfall variability using a high-density rain-gauges network installed in vineyards located in Burgundy (North-East France). Our research aims at understanding whether or not rainfall local variations play a significant role in grape production in quantity and quality.

MATERIALS AND METHODS

Study Area and Rain Gauge Networks

The study was conducted over a 28-km^2 area located in Burgundy winegrowing region, France (**Figure 1**). Climate is oceanic with continental and Mediterranean influences (Chabin, 2004). These influences are represented by moderately cold winter and warm summers, with approximately 760 mm of annual precipitation (Dijon data, 1981–2010 normals[1]). Precipitation is evenly distributed during the year (from 43 to 86 mm each month). During summer, most of the rainfall is brought by thunderstorms, which in hillslope conditions cause a reduced soil water supply compared to recorded rain amount because of their high intensity and the runoff they induce. The terrain of the study area is hilly, due to erosion of a southeast exposed hillside facing a large Plain (Saone Plain) during the Quaternary period. The elevation ranges from 200 to 450 m.

In order to capture space and time variability of rainfall, a very dense rain-gauge network composed of 45 tipping-bucket rain gauges (devices called "Rainnew 111," by Rainwise Inc., Trenton, ME, United States) was installed from 2012 (first tests) to 2014 (final network size). These rain gauges are linked to a Hobo Pendant UA-002-64 event-temperature logger (Onset Computer Corp., Bourn, MA, United States) that records the time of occurrence of bucket tips. The rain gauges, located nearby vineyards, were implemented following the WMO recommendations (World Meteorological Organization, 2008), at a maximum angle of 30° between the top of the gauge to the top of the highest nearest obstacle. In order to assess measurement uncertainty, a pair of two gauges has been installed within a distance of 3 m between each gauge (white filled blue circles on **Figure 1C**). The resolution (0.258 mm/tip) and the average measurement error (ranging from 0.6 to 4.2%) of the rain gauges have been tested during a preliminary study. Network implementation and control are detailed in Pauthier et al. (2014).

[1]https://donneespubliques.meteofrance.fr/

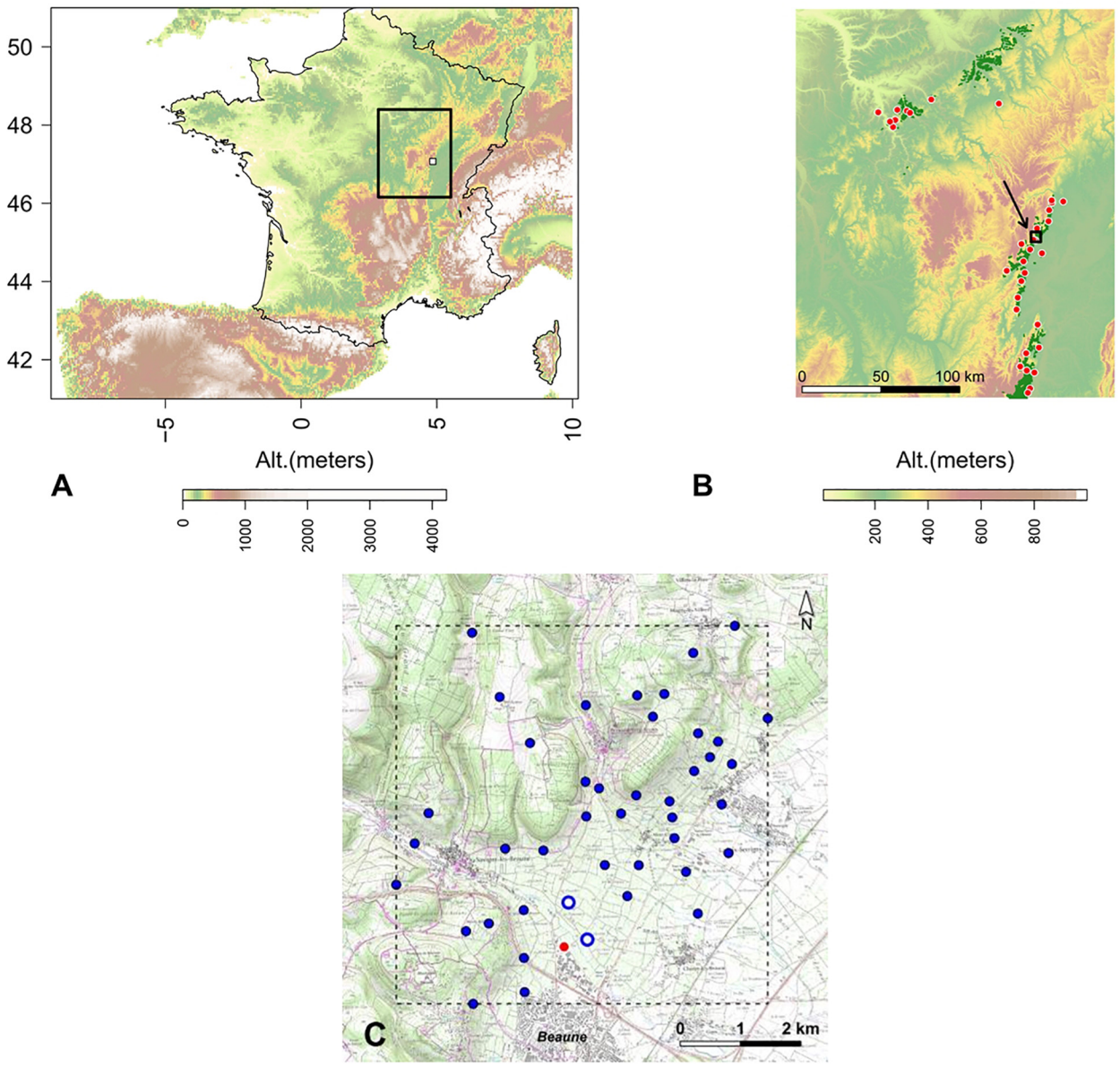

FIGURE 1 | The study area. **(A)** Burgundy wine region location with coordinates in decimal degrees (black box, zoomed in plot **B**); **(B)** Weather stations locations (red dots). Dark-green areas correspond to vineyards. The Hydravitis network location is indicated by a black square pointed by the arrow; **(C)** the Hydravitis high-resolution rain gauges (blue dots) network. White-filled blue dots correspond to location where two rain gauges were installed aside. The red dot shows the location of the Beaune weather station (Climeo mesoscale network).

From 2014 to 2016, during the vegetative periods of the vine, rain gauges were controlled every week in order to limit the potential clogging of the rain gauges.

Despite this very frequent maintenance, clogging, battery, or malfunctioning problems appeared on a few rain gauges. Erroneous or missing data were replaced by spatial interpolation using ordinary kriging.

Mesoscale climate variability was assessed using 2014–2016 data from 34 stations of the Climeo weather network (**Figure 1B**). Climeo is a weather stations network maintained by the union of Burgundy winegrowers and wine merchants (BIVB). It is complemented with stations from the French National Weather service (Meteo–France). All Climeo stations monitor rainfall, air temperature, and humidity 2 m above the ground. Reference evapotranspiration (ET_0) was calculated using the Penman–Montheith FAO-56 formula (Allen et al., 1998). Where solar radiation was not available (27 weather stations), it was estimated using the Hargreaves radiation method (Hargreaves and Samani, 1982). Reference evapotranspiration was estimated by means of the Hargreaves temperature formula

(Hargreaves and Samani, 1985). It was calibrated to match the Burgundy conditions using Penman–Monteith ET_0 as a reference at the seven stations where it was calculated. Hagreaves ET_0 was estimated with a root mean squared difference of 0.55 mm in comparison to Penman–Monteith estimates.

Water Balance Modeling

The Lebon model (Lebon et al., 2003) is based on the geometrical canopy model proposed by Riou et al. (1989) for vertical shoot positioned trellises, coupled to a soil–water balance routine accounting separately for grapevine transpiration and bare soil evaporation. This water balance model requires reference evapotranspiration (ET_0) and rainfall as water inputs, daily solar radiation for solar radiation interception modeling, and daily air temperature for canopy development modeling (crop coefficient) based on degree days. The canopy expands from budburst to 10 days after flowering (estimated to be the date at which the canopy growth is limited due to vegetation mechanical trimming). The vineyard geometry was set for north to south aligned rows (vertical-shoot-position training), with an interrow distance of 1 m, a maximum canopy height of 0.7 m, a maximum canopy width of 0.35 m, and a minimum canopy porosity (proportion of gaps through the canopy) of 0.25. These parameters were set to match usual canopy geometry in the study area.

Temperature and relative humidity data were collected from a weather station located at Beaune (red dot on **Figure 1C**). Solar radiation and wind speed data were taken from a weather station located at Volnay (7.5 km south-eastward of the study area, as these variables were not recorded at Beaune station). ET_0 was calculated using the Penman–Monteith FAO-56 model (Allen et al., 1998).

For all years from 2014 to 2016, 45 runs of Lebon model were performed, with the same parameters and input climate variables except rainfall. For each run, rainfall data collected from a different rain gauge were used. These 45 runs were performed twice, once for soils with water-holding capacity (WHC) set to 50 mm and once for 150 mm.

For each run, the simulated relative grapevine stomatal conductance, derived from the daily fraction of transpirable soil water (FTSW), was used to evaluate the changes in grapevine water status. The simulate grapevine stomatal conductance ranges from 0 (stomata closed, i.e., no transpiration and extreme water deficit stress) to 1 (stomata fully opened, i.e., maximum transpiration and no water deficit stress). Rather than using the bilinear relationship between FTSW and relative grapevine stomatal conductance, we used the inverse exponential equation proposed by Pieri and Gaudillere (2005). This variable is hereafter referred to as water deficit stress index, using WDSI as an acronym. Water deficit stress index was averaged on three major periods of grapevine and grape development period: from budburst (Bud) to flowering (Flo, i.e., blooming), when grapevine primary shoots and leaves actively develop; from flowering to veraison (Ver), when grape develops; and from veraison (Ver) to harvest (Har), when vegetative growth is very low and the grapes ripen.

The phenological stages have been retrieved from weekly observations of a commercial vineyard of *Vitis vinifera* cv. Pinot N from a domain located at Aloxe Corton (in the middle of the study area).

It should be specified that Lebon et al. (2003) model has been developed for flat terrain. It assumes 100% infiltration of precipitation. Although an adaptation to slope conditions has been proposed recently by Hofmann et al. (2014), the Lebon model was preferred because the aim of this study is limited to evaluate the sensitivity of vineyard water balance to the sole local rainfall space and time variability only, for two contrasted soil WHC.

The Lebon model was assessed in the study area by means of grapevine water status and soil water status monitoring to evaluate its relevance to simulate vineyard water balance using year 2013 data from a preliminary research (results not shown).

The results of the first experiment (270 water balance simulations using rainfall collected from 45 rain gauges, for 3 years and 2-soil WHC) were compared to those of a second experiment simulating vineyard water balance spatial variability at mesoscale level using Climeo weather data.

In this second experiment, we followed the same scheme as in the first, whereas all climate parameters (and not only rainfall) from each weather station were used as inputs in the water balance model. Indeed, at mesoscale, using all parameters alike but rainfall would have led to unrealistic weather variables combinations for some days (such as rainfall on a sunny day). As for the (local scale) first experiment, simulations were run for two different soil water capacities (i.e., 50 and 150 mm). This led to 204 water balance simulations (34 weather stations × 3 years × 2 soil WHC). Simulated WDSI was also averaged on three periods of the grapevine vegetative cycle mentioned above: Bud to Flo, Flo to Ver, and Ver to Har.

To avoid confusion throughout the text between time and space variability, the equations and acronyms of different metrics used in this article are defined below. They are statistics calculated for a collection of m (total) locations. At each location j, a rain gauge or weather station is located. The variable X is either rainfall (mm) (acronym = R) or WDSI.

The daily (spatial) range of X for a day i:

$$\delta_{X,i} = max_{j=1}^{m}\left(X_{i,j}\right) - min_{j=1}^{m}\left(X_{i,j}\right) \quad (1)$$

The daily (spatial) standard deviation of X, for a given day i:

$$\sigma_{X,i} = \sqrt{\frac{1}{m}\sum_{j=1}^{m}(X_{i,j} - \bar{X}_i)^2} \quad (2)$$

The daily (spatial) standard deviation of X, for a given period (set of days):

$$\sigma_{X,period} = \sqrt{\frac{1}{m}\sum_{j=1}^{m}(X_{period,j} - \overline{X_{period}})^2} \quad (3)$$

Hereafter, a period is a set of days from a phenological stage to another. For example, σ_{FloVer} is the standard deviation calculated with the X_j values collected at each j location during the flowering-to-veraison (included) period.

$X_{period,\,j}$ can be either the sum of each x_i precipitation record at location (rain gauge or station) j at day i for a collection of n days corresponding to the *period* duration, that is:

$$X_{period,j} = \sum_{i=1}^{n} X_{i,j} \quad (4)$$

or the average of each daily $X_{i,\,i}$ calculated WDSI for location (rain gauge or weather station) j, on a collection of n days corresponding to the *period* duration, that is:

$$X_{period,j} = \frac{1}{n}\sum_{i=1}^{n} X_{i,j} = \overline{X_{period,j}} \quad (5)$$

The *period* acronym can either be *BudFlo*, the budburst to flowering period; *FloVer*, the flowering to veraison period; or *VerHar*, the veraison to harvest period.

The δ_i, σ_i, and σ_{period} are metrics of the spatial variability of rainfall or WDSI at local or at mesoscale.

RESULTS

Local Variability of Rainfall

The 3 years exhibited different profiles in climate conditions during the vegetative season (**Table 1**).

2014 was cooler than average during the vegetative cycle of grapevine, with a wet summer (**Table 1** and **Figure 2**). As the late winter of 2014 was quite warm, budburst occurred rather early (April 4), which, together with a warm period in the first two decades of June, lead to a harvest date on September 10, near the average of the normals (1986–2015; see **Table 1**). 2015, in contrast, was much warmer and dryer than average, with a little rainfall until harvest but two storms events in mid-June. All phenological stages in 2015 were early, in comparison to 2014 and 2016, from 2 (flowering) to 20 (veraison) days. In 2016, spring rainfall was high. Because of a cool and wet spring, flowering date (June 21) occurred 2 weeks later than in 2014 and 2015. Veraison was also considerably delayed (10 days later than in 2014 and 20 days later than in 2015). A warm spell from late August to early September 2016 allowed to reach maturity on September 25.

Spatial variability of rainfall at daily time step was largest during heavy daily rainfall events (i.e., showers and storms), with a maximum range on May 9, 2016 (42.1 mm), and on July 22, 2016 (42 mm). Not surprisingly, local variability of rainfall is larger on heavy precipitation events (**Figure 3A**). For a single day, local daily rainfall can range up to 42 mm.

In **Figure 3B**, lines are mapped to the x axis and the left y axis, whereas dots (in different shapes according to the year) are mapped to x axis and right y axis. Lines in **Figure 3B** show that 50% of the total cumulated rainfall from budburst to harvest (gray dashed horizontal line) is provided by 6% (in 2015) to 9% (in 2014) of the rainy days (colored number on the top of **Figure 3B**). Dots in **Figure 3B** show that during these days, the spatial variability is large: standard deviation between all 45 rain gauges is always larger than 1 mm; it is frequently greater than 2 mm and can reach up to 11 mm (in 2016). The fact that a few heavy rainy days, characterized by large local spatial variation, control most of the water input during the grapevine growing season might induce substantial variation in soil–water balance at local scale level.

Water Balance Variability at Local Scale

Figure 4 shows water balance modeling across the rain-gauge locations for all years, under the assumption of 100% precipitation infiltration. In 2014 and 2015, early water deficit was observed, with large spatial differences in WDSI profiles on soils with low WHC (50 mm) in 2014. The frequent rainfall events during summer 2014 reduced water deficit in both low and high WHC soils, where moderate to no water deficit was simulated at harvest. During 2015, after a series of 3 rainy days on June 12 (average = 11.7 mm), 14 (14.8 mm), and 15 (16.16 mm), the absence of rainfall until late July induced moderate to severe water deficit during most of the flowering to veraison period. In August, a series of rainy events brought heterogeneous rainfall in space, maintaining very high stress at a few locations, whereas most of the local area was sufficiently fed to reduce water deficit. Water deficit stress index at harvest was below 0.3 (i.e., moderate to severe) at almost all rain-gauges locations.

In 2016, water deficit installed quickly after flowering for 50 mm WHC, whereas it dropped gradually until mid-September to reach severe water deficit in mid-September on soils with moderate water capacity (i.e., WHC = 150 mm). A wet spell from September 15 to 19 reduced simulated water stress until harvest on September 25.

The spatial structure in grapevine water deficit stress is not maintained in time within the same year. That is, during a given year, areas with the lowest WDSI can change. In 2015, the highest simulated water deficit caused by rainfall spatial variability was located in the northern hilly part of the study area during the flowering to veraison period (**Figure 5**, top-right corner), whereas it was located on the southeastern part of the study area during the veraison-to-harvest period (simulations for a 50-mm WHC soil). In 2016, the location where the lowest flowering-to-veraison average WDSI (0.17) was calculated is at the northeast part of the study area, whereas it is found in the southwestern part of the study area for veraison to harvest (lowest WDSI = 0.19).

Local to Mesoscale Water Balance Variability

Water deficit stress index spatial variability at local scale (induced by changes in rainfall spatial distribution) was compared to climate-induced WDSI spatial variability at meso-scale, i.e., the Burgundy wine–producing region, using Climeo weather stations network. At local scale, the Hydravitis network captures rainfall variability over 6.3 (from north to south) and 6.1 (from west to east) km. In contrast, the Climeo network captures climate variable from 186 (from north to south) to 116 (west to east) km.

Figure 6 presents the distributions of WDSI averaged over three grapevine development periods: from budburst to flowering, from flowering to veraison, and from veraison to

TABLE 1 | Phenological stages and phases (period during two stages) dates, durations, *number of days*, and corresponding cumulated rainfall and average temperature.

		Budbreak	B–F	Flowering	F–V	Veraison	V–H	Harvest	B–H
Dates	2014	4-Apr	*66*	8-Jun	*69*	15-Aug	*27*	10-Sep	159
Duration	2015	12-Apr	*56*	6-Jun	*61*	5-Aug	*34*	7-Sep	148
	2016	12-Apr	*71*	21-Jun	*66*	25-Aug	*32*	25-Sep	166
	1986–2015	**14-Apr**	*59*	**11-Jun**	*64*	**12-Aug**	*35*	**15-Sep**	**154**
Rainfall (mm)	2014	-	61	-	243	-	29	-	333
	2015	-	97	-	82	-	59	-	237
	2016	-	254	-	114	-	34	-	402
	1986–2015	-	**130**	-	**131**	-	**67**	-	**329**
Temperature (average) (°C)	2014	-	14	-	20.2	-	17.6	-	17.2
	2015	-	15.4	-	22	-	20	-	19.1
	2016	-	13.9	-	20.7	-	20.3	-	17.8
	1986–2015	-	**14.8**	-	**20.7**	-	**19.1**	-	**18.1**

B–F, budburst to flowering period; F–V, flowering to veraison period; V–H, veraison to harvest period; B–H, budburst to harvest period. Colors within the table highlight dry (yellow tone) to wet (blue tone) or cool (blue tone) or warm (red tone) periods, in comparison to those during the 2014–2016 period. Phenological dates correspond to Vitis vinifera cv. Pinot noir phenological observations from a winery near Beaune; climate data correspond to the Meteo–France/Climeo Beaune weather station (red dot in ***Figure 1C****). Values in bold correspond to the 1986–2005 average.*

harvest. From budburst to floraison, WDSI remained high, so that little spatial variability was observed, either at local or at mesoscale, as soil water content remained close to the soil WHC, thus maintaining WDSI close to its maximum value. In 2014, however, weak water deficit was simulated at both local and mesoscale level for soil with low WHC (i.e., 50 mm). From flowering to veraison, water deficit was larger on low WHC soils, which was not the case during veraison to harvest, when scarce rainy events refill most of the soil WHC in low WHC soils.

Moderate to severe water deficits were observed during the flowering-to-veraison period in 2015 and from veraison to harvest in 2016.

At mesoscale level, larger spatial variability was observed during the veraison-to-harvest period than during other periods, in all 3 years studied. Spatial standard deviation ($\sigma_{WDSI,\ period}$) ranged from 0.1034 (year 2015, soil WHC = 150 mm) to 0.1835 (year 2014, soil WHC = 50 mm, **Table 2**). In 2014, climate-based water deficit simulations suggested that grapevine water stress ranged from severe to moderate water deficit up to no water deficit, following the classification proposed by van Leeuwen et al. (2009).

In general, WDSI spatial variations were larger at mesoscale than at local scale (**Figure 6**). To compare spatial variation within each development period at both spatial scales, we calculated the ratio between local and mesoscale WDSI standard deviations, i.e., (local σ_{period})/(mesoscale σ_{period}) in **Table 2**. In all cases (i.e., year per period per WHC) but one, this ratio is lower than one, indicating that mesoscale climate variability induces more variation in WDSI than local scale rainfall does (**Table 2**). In three cases, however, the difference in WDSI variance was not significant (Bartlett variance comparison test, at $\alpha = 0.05$): during the budburst-to-flowering period for both WHC soils in year 2016 and from flowering to veraison for the 150-mm WHC soil in year 2016. The difference in standard deviation for the 2016 budburst-to-veraison period, although significant, is almost null, because WDSI was close to one at all locations and at both local and mesoscale. From flowering to veraison during 2016 for a 150-mm WHC soils, the standard deviation was 0.0519 at local scale and 0.0645 at mesoscale level. The local scale spatial variability was as large as 81% of the mesoscale spatial variability during this period (see the "ratio%" column in **Table 2**).

DISCUSSION

This article measured the spatial variability of rainfall and estimated the relevance of this phenomenon on the grapevine water status during 3 years. Variation in rainfall was measured to be as large as 43 mm in a single rain event. To evaluate the potential physiological significance of the rainfall variability on grapevine water status, the model developed by Lebon et al. (2003) was used, because it is commonly used in viticulture, and it has shown its efficacy in many viticultural conditions (e.g., Pellegrino et al., 2006; Celette et al., 2010). It is simple to use and produce meaningful outputs to both grape growers and researchers. This model does not use an absolute relationships to link soil water status and grapevine water deficit. In the model, plant water deficit stress depends on the water availability relative to the soil WHC, that is, the FTSW. Water deficit stress index is related to FTSW through an inverse exponential function that led to a rapid decrease in WDSI when FTSW is below 0.4 (Pieri and Gaudillere, 2005). This can sometimes bring to counterintuitive results, when water available to plants is low, rainfall events might be sufficient to refill most of the soil capacity on low WHC soils, whereas they might refill only partly the soil water capacity of higher WHC soils. In this case, the model will simulate higher plant water stress for high WHC soil than for low WHC soil, because in the first case the FTSW would be higher. For example, in 2015, the average WDSI was 0.02 on August 3 for 50-mm SHC soils (2 days before veraison, see **Figure 4**), whereas it was slightly higher on 150 SHC soils (average WDSI = 0.04). On August 4, 9 mm of rainfall refilled about 17% of the 50-mm WHC soil, whereas only 6% of the 150-mm WHC soil was brought by this rainfall amount. Consequently, the WDSI of the 50-mm WHC soil was, respectively, more than twice as high (0.58) than the

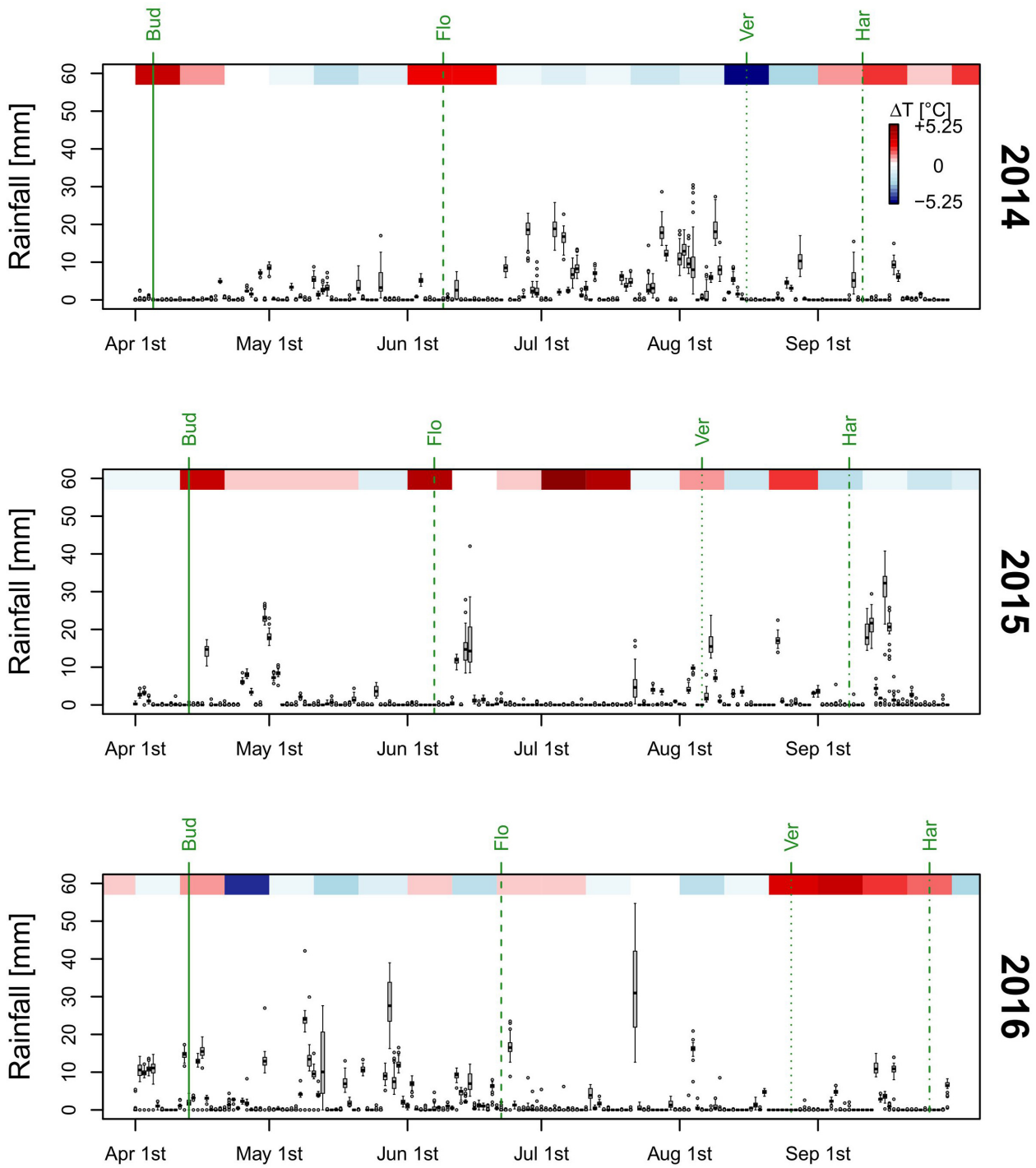

FIGURE 2 | Daily rainfall variability. Each box plot shows the distribution of daily rainfall collected by 45 rain gauges. The colored bar on the top of each plot indicates the anomaly in average temperature [ΔT (°C)] per decade (i.e., 10 days) in comparison to the 1986–2015 decade average, from Beaune weather station (red dot on **Figure 1C**).

150-mm WHC soil WDSI (0.27). While these situations are not frequent, in the 3 years studied here, they finally led to higher simulated water stress on 150-mm WHC than on the 50-mm WHC soil from veraison to harvest in 2015 and 2016 (at local scale level). This model is constantly under development, it has been improved by Celette et al. (2010) to account for the presence of cover crop and then by Hofmann et al. (2014) to account for slope effect on vineyard radiative balance. However, the

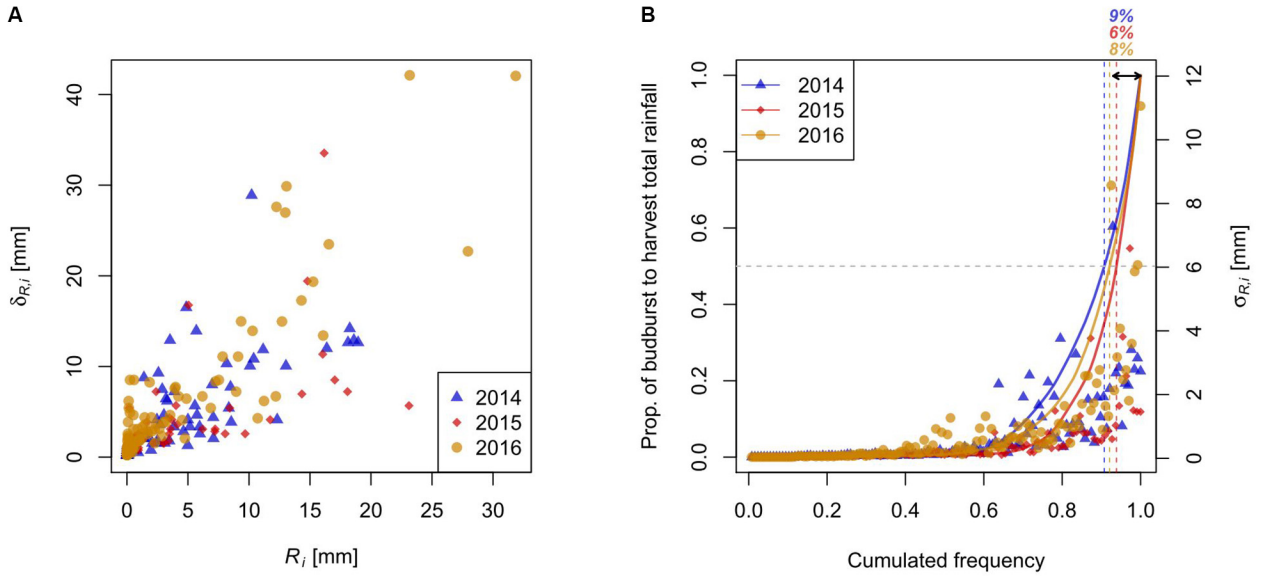

FIGURE 3 | Local rainfall variability at daily time step during the grapevine vegetative period. **(A)** Spatial precipitation range ($\delta_{R,i}$, i.e., max.–min. rainfall collected by the rain gauges on a given day) as function of daily precipitation height (R_i, mm) averages the Hydravitis local scale network. **(B)** The lines show the proportion of total cumulated rainfall from budburst to harvest as function of the cumulated distribution of each rainy (>0 mm) event (i.e., day), and the dots show the daily standard deviation of rainfall ($\sigma_{R,i}$, i.e., daily spatial variability). The colored number on the top of the plot indicates the percentage of rainy days (range underlined by arrows) during the budburst to harvest period that bring 50% of the total cumulated rainfall for this period (area delineated by colored dashed vertical lines).

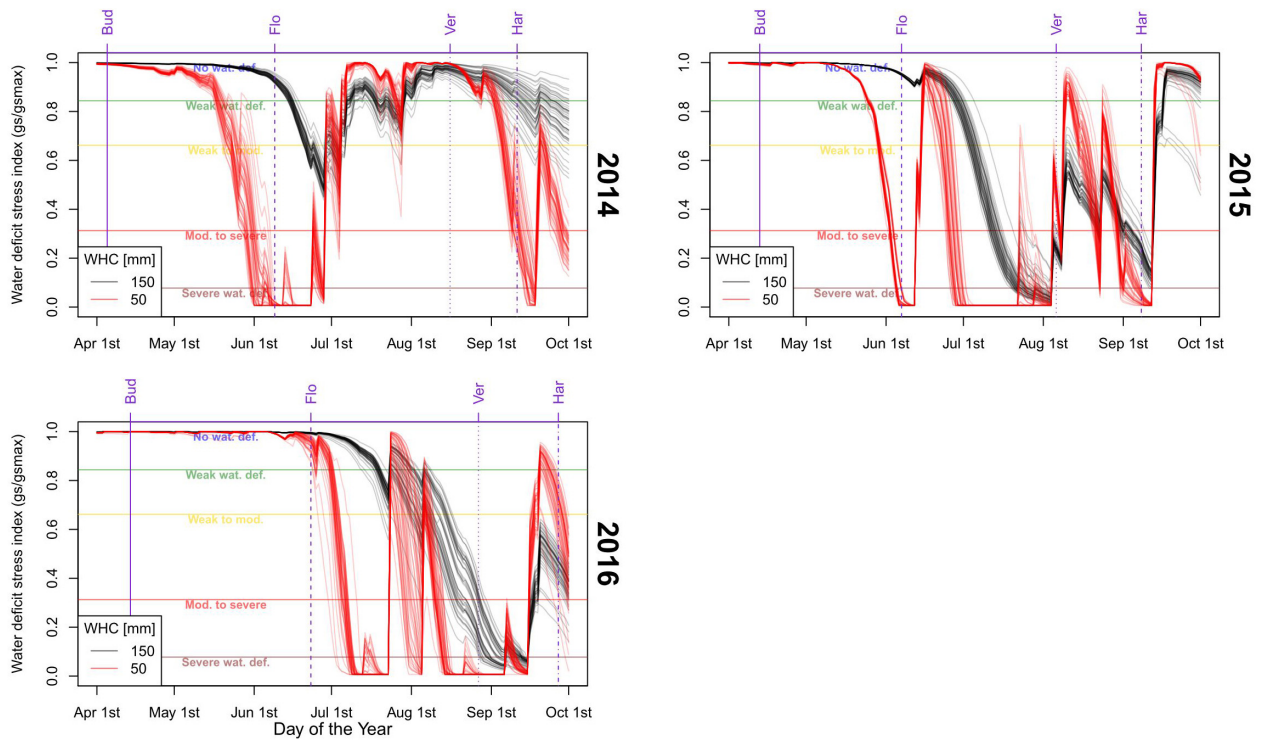

FIGURE 4 | Water deficit stress index daily values for low (50 mm) and high (150 mm) WHC soils. Each line corresponds to a simulation using rainfall value collected at one of the 45 rain gauges of Hydravitis local scale network. Threshold values between each class have been calculated from corresponding predawn leaf water potential values in table 1 of van Leeuwen et al. (2009), using the exponential relationship between fraction of transpirable soil water and predawn leaf water potentiel (Figure 3 in Lebon et al., 2003). The colored horizontal lines indicates the upper limit of water deficit classes as proposed by van Leeuwen et al. (2009).

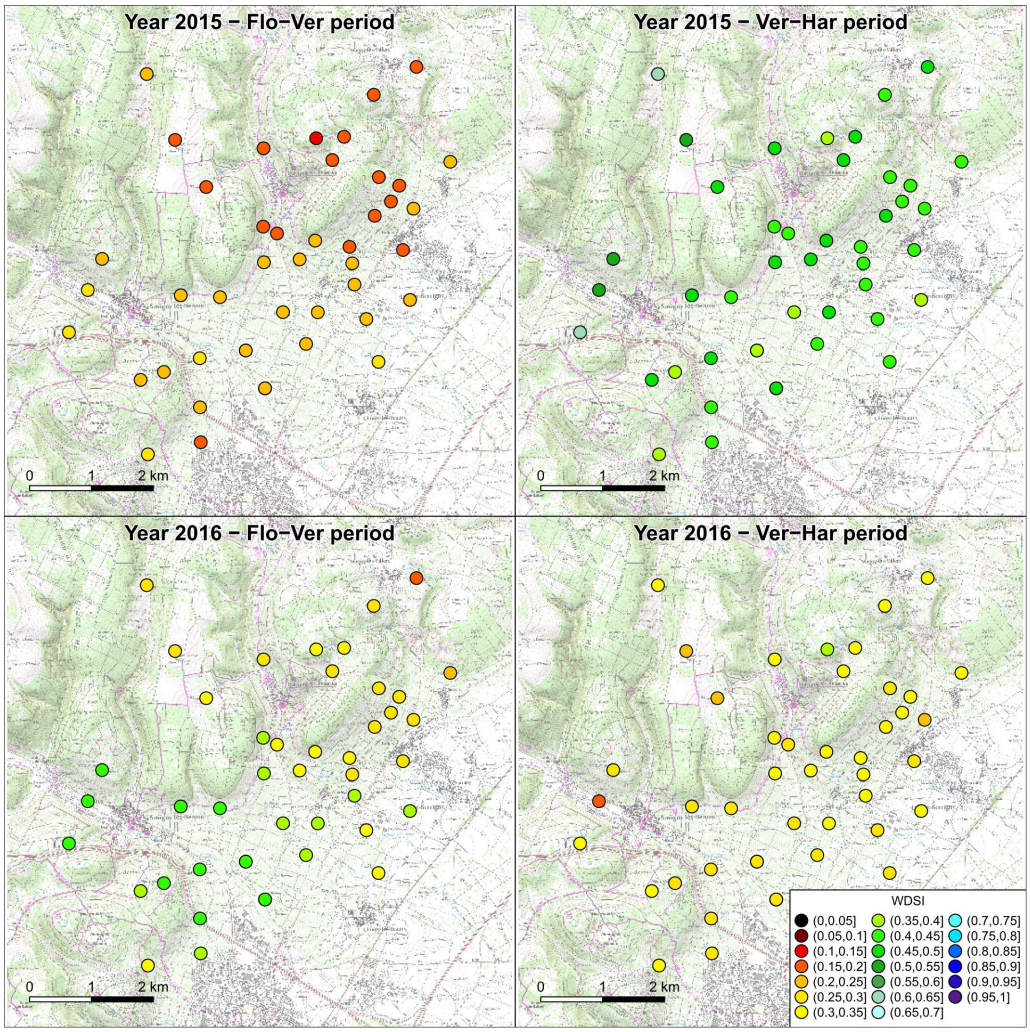

FIGURE 5 | Maps of the average WDSI calculated using a 50-mm WHC soil during two grapevine development stages (Flo–Ver, flowering to veraison; Ver–Har, veraison to harvest) in 2015 and 2016. Each point corresponds to a rain gauge of the Hydravitis local scale network.

relationships between soil water availability and plant water status have never been modified from the one in the original model. In our opinion, a better understanding of the grapevine soil–water relationships is needed and deserves further investigations from soil scientists and plant ecophysiologists.

Variability in vineyard water status can be caused by many factors: soil, terrain, plant material, training system, soil management, and climate. This article makes abstraction of all other factors except soil WHC, to concentrate on the effect of rainfall variability on plant water status. The soil WHCs that were used in this study (50 and 150 mm) are comparable to the upper and lower limits of WHC range previously measured (from 42 to 176 mm) in Burgundy vineyards, at nearby locations (Curmi et al., 2012).

Our results show that at local scale the difference in plant water status caused by the physical environment is related more to soil variability than to rainfall variability. As shown in **Figure 4**, differences in WDSI are greater between the two soil scenarios than for a given WHC because of differences in rainfall. Differences in soils affect not only the magnitude of the water stress but also the time of occurrence. However, differences in water status between the soil scenarios depend on the meteorological conditions, and they manifest only when rainfall is limited, and differences are not observable at the beginning of the season. Within the study area (see **Figure 1C**), Brillante et al. (2016a) monitored two grapevine cv Chardonnay grafted on SO4 rootstock subplots, 40 m apart on the same slope, in the same commercial vineyard plot. Stem water potential (Ψ_{stem}) measured from two consecutive summers differed from 0.2 MPa on average (with maximum of 0.4 MPa) to 0.1 MPa between the two locations and was largely correlated to soil water content variations. Differences were most probably the result of changes in soil and subsoil characteristics (e.g., depth, texture, gravel content. . .) or/and rainfall runoff, and probably little affected by changes in rainfall inputs, as both locations compared in their study were 40 m apart. Rainfall spatial variability at

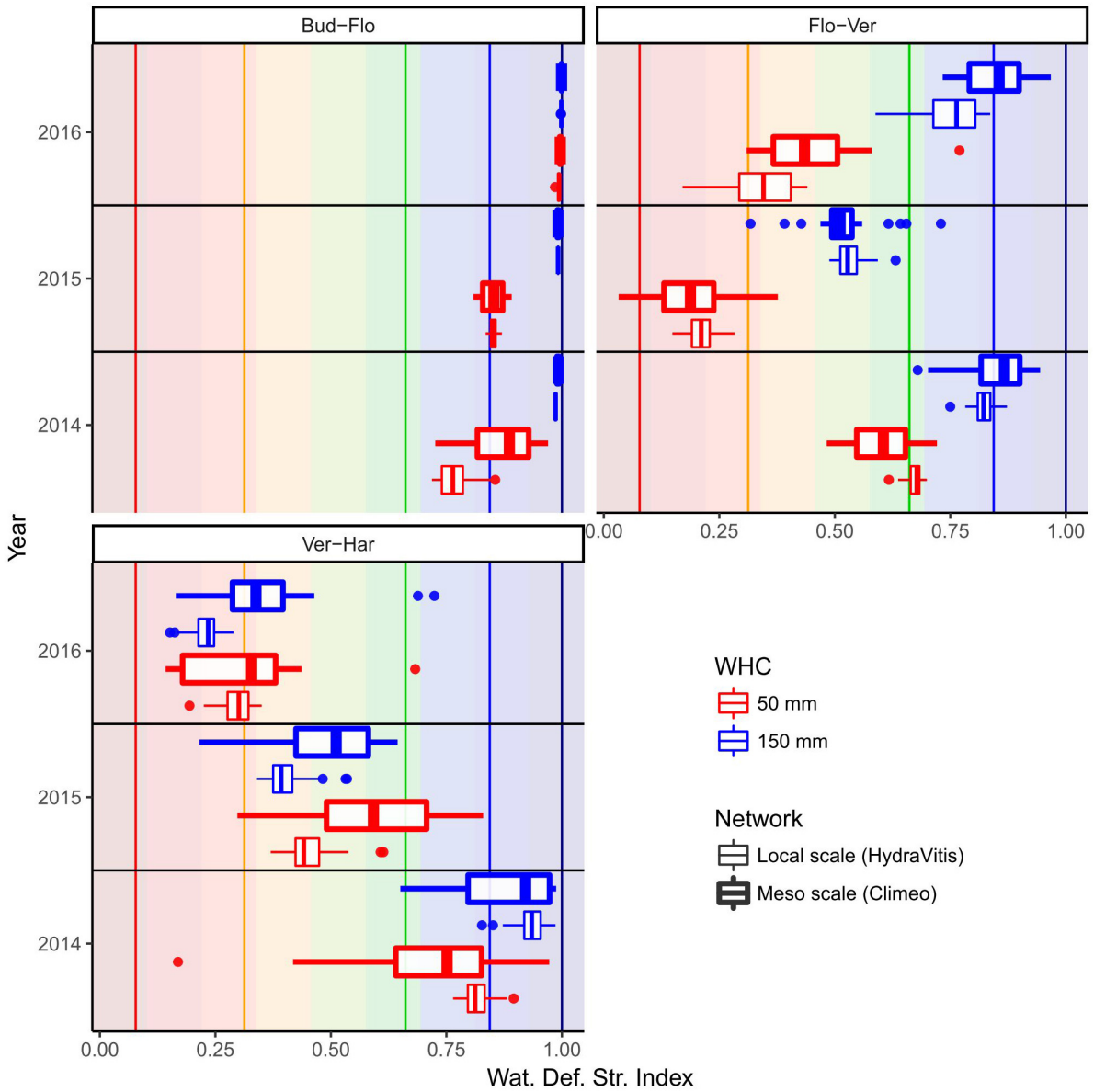

FIGURE 6 | Comparison of local to mesoscale variability of simulated WDSI daily averages during three development stages (Bud–Flo, budburst to flowering; Flo–Ver, flowering to veraison; Ver–Har, veraison to harvest), for soils with low (50 mm) and high (150 mm) soil water contents (WHC). Note that local variability of WSDI resulted from simulations using different rainfall data at each location, whereas all other climate-related input (relative humidity, air temperature, wind speed, and solar radiation) came from the same location. For mesoscale variability, simulations were made using different climate related input for each location.

local scale level might either enhance or buffer the soil-induced differences on observed grapevine water status. In an empirical model developed in the study area, Brillante et al. (2016b) showed that amounts of rainfall lower than 10 mm in the previous 7 days would linearly reduce Ψ_{stem} of 0.02 MPa per mm (average across soil and weather conditions).

The water balance sensitivity was tested at mesoscale level using all climate parameters variations between weather stations, and not rainfall only, contrarily to the local scale water balance modeling experiment. It would have been unrealistic to simulate water balance modeling over the whole Burgundy wine region using the same climate data but rainfall from each weather station, as nonsense data combination would have occurred, such as rain in sunny weather (i.e., high solar radiation and evapotranspiration) conditions. Consequently, mesoscale water balance modeling simulates changes in both grapevine development timing (leaf area and solar radiation interception by rows) and evapotranspiration, whereas these major components of the water balance are kept even at local scale. Spatial variation of rainfall at local scale level induced changes in WDSI, as large as 15–102% of the climate-induced spatial variability at mesoscale level. During the flowering-to-veraison period in 2016,

TABLE 2 | Average (mean) and standard deviations ($\sigma_{WDSI,\ period}$) of mean WDSI during three development periods of grapevine, in 2014, 2015, and 2016.

			Mean		$\sigma_{WDSI,\ period}$			Variance diff.	
Stage	**Year**	**WHC**	**Local**	**Meso**	**Local**	**Meso**	**Ratio (%)**	***P*-value**	**Sign**
Bud–Flo	2014	50	0.77	0.87	0.0330	0.0709	47	2.9E-05	***
		150	0.99	0.99	0.0017	0.0032	54	8.1E-04	***
	2015	50	0.85	0.85	0.0085	0.0254	34	3.1E-09	***
		150	0.99	0.99	0.0003	0.0014	20	4.9E-17	***
	2016	**50**	**1.00**	**1.00**	**0.0018**	**0.0017**	**102**	9.0E-01	–
		150	**1.00**	**1.00**	**0.0003**	**0.0003**	**97**	8.7E-01	–
Flo–Ver	2014	50	0.67	0.60	0.0172	0.0725	24	1.9E-14	***
		150	0.82	0.85	0.0234	0.0720	32	1.1E-09	***
	2015	50	0.21	0.18	0.0294	0.0870	34	3.7E-09	***
		150	0.53	0.52	0.0285	0.0894	32	6.1E-10	***
	2016	50	0.38	0.45	0.0656	0.1124	58	3.4E-03	**
		150	**0.80**	**0.85**	**0.0519**	**0.0645**	**81**	2.5E-01	–
Ver–Har	2014	50	0.82	0.72	0.0280	0.1835	15	7.4E-22	***
		150	0.93	0.88	0.0328	0.1136	29	2.2E-11	***
	2015	50	0.45	0.59	0.0516	0.1367	38	1.1E-07	***
		150	0.40	0.50	0.0432	0.1034	42	1.8E-06	***
	2016	50	0.30	0.30	0.0340	0.1333	26	2.9E-13	***
		150	0.25	0.37	0.0366	0.1342	27	2.9E-12	***

*WHC, soil water-holding capacity (mm); Local, local scale level simulations (Hydravitis network); Meso, mesoscale level simulations (Climeo network); Ratio, $\sigma_{local}/\sigma_{meso}$, i.e., the ratio between local scale to mesoscale standard deviations; Variance diff., the results of the Bartlett test of variance homogeneity, with its p value and significance code (***$p \leq 0.001$; **$p \leq 0.01$ and *$p \leq 0.05$). The values in bold correspond to periods/years for which local WDSI variance if not statistically significant ($p > 0.05$) mesoscale WDSI variance.*

simulated WDSI was as variable in space at local scale as at regional levels.

Within vine growing regions of similar size (about 28 km^2, i.e., 2,800 ha), changes in soil management, plant material, training systems, terrain and climate parameters might also either cumulate or compensate the potential changes in vineyard water status as simulated in our study. Lopes et al. (2011) compared the consequences of soil tillage and permanent resident vegetation coverage on soil and grapevine cv. Tempranillo water status in Alentejo (southern Portugal) wine region. Significant differences in FTSW and predawn leaf water potential (Ψ_{PD}) were observed within the two soil management systems. Ψ_{PD} commonly differed from about 0.1 MPa between the two compared systems, where the soil tillage showed most of the time lower water deficit. Celette (2007) observed up to about 0.3 MPa differences in Ψ_{PD} when comparing several interrow grass cover to bare soil on 5- to 7-year-old grapevine cv. Aranel grated with Fercal rootstock. Marguerit et al. (2012) studied the control of scion (cv. Cabernet-Sauvignon) water use from 138 *V. vinifera* × *Vitis riparia* rootstock genotypes. While metrics are not comparable to our study, they showed that rootstock material can strongly affect water status of grapevine. For example, scion relative transpiration rate (i.e., WDSI) can show difference up 0.63, depending on the rootstock used. Training systems, through changes in microclimate and incoming solar radiation interception by grapevine (Pieri and Gaudillère, 2003), can considerably affect vineyard transpiration and water use efficiency (Baeza et al., 2005; Reynolds and Heuvel, 2009).

In our study, we focused mainly on rainfall daily depth without accounting for rainfall intensity, which can dramatically affect the water input in soil, depending on runoff.

Runoff is wrongfully often not accounted in water balance modeling.

Yet, it can strongly affect soil water refill. Gaudin et al. (2010) showed that off-season soil water refilling might not be achieved according to rainfall intensity, topography, and soil management (bare soil or interrow grass cover). Biddoccu et al. (2017) observed a reduction of vineyard soil surface runoff of 63% when soil was covered with grass, in comparison to conventional tillage, leading to changes in soil water content. Besides, as runoff is also related to soil permeability; thus, humidity and rainfall spatial variation might affect soil water status through changes in runoff, depending on soil surface texture, structure, and humidity.

Runoff depends on terrain characteristics, among which slope intensity and position. However, slope impact on runoff is not straightforward, as various factor can interact and modify the impact of slope on runoff (Fang et al., 2008). Under intense rainfall, runoff measurements based on simulated rainfall in Mediterranean vineyards showed no impact concerning the slope position on runoff (Cerdà and Rodrigo-Comino, 2020). As runoff is very sensitive to various parameters, its simulation is unsatisfactory when broadly estimated (Chahinian et al., 2005). The data set used in this study could be used in further work to better assess, through hydrological modeling, the potential impact of local rainfall variability on runoff and erosion. Indeed, erosion is a major physical process that leads

to land degradation, and soil preservation is a key element of Sustainable Development Goals (Keesstra et al., 2018). As often planted in slopes, grape is a crop for which soil erosion is a major concern (Rodrigo-Comino et al., 2018) and can be considerable in steep slope cool climate grape growing areas such as Burgundy and Germany (Quiquerez et al., 2014; Rodrigo Comino et al., 2016).

Over the study area of our research, terrain might also greatly affect water balance by modifying solar incoming radiation and evapotranspiration. The Lebon et al. (2003) water balance model was strongly enhanced by Hofmann et al. (2014) to account for slope effect on vineyard radiative balance. Their research showed good agreement between simulated and measured FTSW on three plots planted with cv Riesling over the same hillslope in Germany (Rüdesheim region). It suggests that solar radiation partitioning from interrow and row, together with modification in potential (or reference) evapotranspiration by terrain solar radiation interception, soil characteristics, soil management, and training systems variations, influences the changes in grapevine water dynamics. Unfortunately, the study does not compare the relative contribution of these factors to differences in vineyard water status between the validation plots.

Terrain, soil, training systems, and plant material are rather stable through time. When studying a wine producing region, potential water status of grapevine is therefore inferred through these vineyard characteristics. Our simulations indicate that the impact of these vineyard characteristics on plant water status can differ according to local rainfall variations in time and space. According to the water balance modeling performed in our study, a lower soil water capacity can lead to, in a counterintuitive manner, lower water deficit. Water balance simulation in 2015 shows a lower WDSI (i.e., higher water deficit related stress) on 150-mm WHC soils than on 50-mm WHC soils from veraison to harvest (**Figure 6**, bottom), as rainy events refilled most of the WHC on low WHC soils, whereasthe FTSW remained low on high WHC soils. In the study area, rainfall spatial distribution strongly changes over time. During the three vintages during which rainfall spatial distribution was monitored, no location was preferentially wetter than others were. Consequently, rainfall modifies the zoning of lower or higher water deficit in the study area through time, so that even if several parameters favor a specific water status (e.g., water deficit promoting factors such as low WHC soils, steep slope, considerable leaf area. . .), the rather odd spatial distribution of rainfall might partially change the spatial distribution of grapevine water deficit at local scale level.

For viticulture, soil is often considered as the sole environmental component that explains grape water status variations and thus the major factor of terroir. Our results show that when comparing spatial structure of grapevine water status at local scale, one should account for the impact of rainfall local variability.

CONCLUSION

This article shows that spatial variability in rainfall could probably affect grapevine physiology independently from soil or other factors. Differences in rainfall did not have specific spatiotemporal structures in the years and at the scale of this study. Further investigations and sensitivity tests of grapevine water balance modeling to climate, plant material, soil physicochemical properties, soil management, training system, and terrain to better represent the viticultural system diversity would probably be of great interest. It would provide useful contribution to achieve a better understanding of plant water status response to the environment.

To the best of our knowledge, no studies reporting the impact of rainfall spatial variation at local scale on crops has been reported. The issues of rainfall spatial variability impact on small catchment in agriculture are numerous: plant protection, crop quality, mechanization, and soil conservation. The progress in high-resolution radar-aided precipitation detection makes it an interesting source of data to address agricultural water management at local scale level.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

BP collected and prepared the data. BB and BP analyzed the data. BB wrote the first draft. BP, BB, LB, OM, JL, CV, TC, and YR finalized the manuscript. All authors contributed to the article and approved the submitted version.

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REFERENCES

Acevedo-Opazo, C., Ortega-Farias, S., and Fuentes, S. (2010). Effects of grapevine (*Vitis vinifera* L.) water status on water consumption, vegetative growth and grape quality: an irrigation scheduling application to achieve regulated deficit irrigation. *Agric. Water Manag.* 97, 956–964. doi: 10.1016/j.agwat.2010.01.025

Ali, M. H., and Talukder, M. S. U. (2008). Increasing water productivity in crop production—A synthesis. *Agric. Water Manag.* 95, 1201–1213. doi: 10.1016/j.agwat.2008.06.008

Allen, R. G., Pereira, L. S., Raes, D., and Smith, M. (1998). *Crop Evapotranspiration: guidelines for Computing Crop Water Requirements*. Rome: FAO.

André, F., van Leeuwen, C., Saussez, S., Van Durmen, R., Bogaert, P., Moghadas, D., et al. (2012). High-resolution imaging of a vineyard in south of France using

ground-penetrating radar, electromagnetic induction and electrical resistivity tomography. *J. Appl. Geophys.* 78, 113–122. doi: 10.1016/j.jappgeo.2011.08.002

Baeza, P., Ruiz, C., Cuevas, E., Sotés, V., and Lissarrague, J.-R. (2005). Ecophysiological and agronomic response of tempranillo grapevines to four training systems. *Am. J. Enol. Vitic.* 56, 129–138.

Bellvert, J., Zarco-Tejada, P. J., Girona, J., and Fereres, E. (2013). Mapping crop water stress index in a 'Pinot-noir' vineyard: comparing ground measurements with thermal remote sensing imagery from an unmanned aerial vehicle. *Precis. Agric.* 15, 361–376. doi: 10.1007/s11119-013-9334-5

Berne, A., Delrieu, G., Creutin, J.-D., and Obled, C. (2004). Temporal and spatial resolution of rainfall measurements required for urban hydrology. *J. Hydrol.* 299, 166–179. doi: 10.1016/j.jhydrol.2004.08.002

Biddoccu, M., Ferraris, S., Pitacco, A., and Cavallo, E. (2017). Temporal variability of soil management effects on soil hydrological properties, runoff and erosion at the field scale in a hillslope vineyard. *North-West Italy. Soil Tillage Res.* 165, 46–58. doi: 10.1016/j.still.2016.07.017

Bonfante, A., Agrillo, A., Albrizio, R., Basile, A., Buonomo, R., De Mascellis, R., et al. (2015). Functional homogeneous zones (fHZs) in viticultural zoning procedure: an Italian case study on Aglianico vine. *Soil* 1, 427–441. doi: 10.5194/soil-1-427-2015

Bonnefoy, C., Quenol, H., Bonnardot, V., Barbeau, G., Madelin, M., Planchon, O., et al. (2013). Temporal and spatial analyses of temperature in a French wine-producing area: the Loire Valley. *Int. J. Climatol.* 33, 1849–1862. doi: 10.1002/joc.3552

Brillante, L., Bois, B., Lévêque, J., and Mathieu, O. (2016a). Variations in soil-water use by grapevine according to plant water status and soil physical-chemical characteristics—A 3D spatio-temporal analysis. *Eur. J. Agron.* 77, 122–135. doi: 10.1016/j.eja.2016.04.004

Brillante, L., Mathieu, O., Lévêque, J., and Bois, B. (2016b). Ecophysiological modeling of grapevine water stress in burgundy terroirs by a machine-learning approach. *Front. Plant Sci* 7:796. doi: 10.3389/fpls.2016.00796

Celette, F. (2007). *Dynamique des Fonctionnements Hydrique et azoté dans une vigne Enherbée sous le Climat Méditerranéen*. Available at: http://www.agro-montpellier.fr/theses/extranet/07-0009_Celette.pdf (accessed December 11, 2016).

Celette, F., Ripoche, A., and Gary, C. (2010). WaLIS—A simple model to simulate water partitioning in a crop association: The example of an intercropped vineyard. *Agric. Water Manag.* 97, 1749–1759. doi: 10.1016/j.agwat.2010.06.008

Cerdà, A., and Rodrigo-Comino, J. (2020). Is the hillslope position relevant for runoff and soil loss activation under high rainfall conditions in vineyards? *Ecohydrol. Hydrobiol.* 20, 59–72. doi: 10.1016/j.ecohyd.2019.05.006

Chabin, J.-P. (2004). L'excellence aux limites. . . ou le paradoxe des vignobles septentrionaux français d'après l'exemple côte-d'orien. Revue Géographique de l'Est 44, 9–16.

Chahinian, N., Moussa, R., Andrieux, P., and Voltz, M. (2005). Comparison of infiltration models to simulate flood events at the field scale. *J. Hydrol.* 306, 191–214. doi: 10.1016/j.jhydrol.2004.09.009

Ciach, G. J., and Krajewski, W. F. (2006). Analysis and modeling of spatial correlation structure in small-scale rainfall in Central Oklahoma. *Adv. Water Resour.* 29, 1450–1463. doi: 10.1016/j.advwatres.2005.11.003

Clothier, B., Dierickx, W., Oster, J. D., Perry, C. J., and Wichelns, D. (2010). Investing in water for food, ecosystems, and livelihoods. *Compr. Assess. Water Manag. Agric.* 97, 493–494. doi: 10.1016/j.agwat.2009.11.001

Condon, A. G., Richards, R. A., Rebetzke, G. J., and Farquhar, G. D. (2004). Breeding for high water-use efficiency. *J. Exp. Bot.* 55, 2447–2460. doi: 10.1093/jxb/erh277

Curmi, P., Ayachi, S., Taoutaou, S., Louamba, J., Brayer, J.-M., Guerinot, M., et al. (2012). *Characterization of Water Reserve of the Burgundy vineyard soils in the Monitoring of Grape Maturation and for Understanding the Epidemiology of Wood Diseases*. Dijon: Université de Bourgogne, 435–437.

de Fraiture, C., and Wichelns, D. (2010). Satisfying future water demands for agriculture. *Compr. Assess. Water Manag. Agric.* 97, 502–511. doi: 10.1016/j.agwat.2009.08.008

Deloire, A., Carbonneau, A., Wang, Z., and Ojeda, H. (2004). Vine and water: a short review. *OENO One* 38, 1–13. doi: 10.20870/oeno-one.2004.38.1.932

Duncan, M. R., Austin, B., Fabry, F., and Austin, G. L. (1993). The effect of gauge sampling density on the accuracy of streamflow prediction for rural catchments. *J. Hydrol.* 142, 445–476. doi: 10.1016/0022-1694(93)90023-3

Fang, H. Y., Cai, Q. G., Chen, H., and Li, Q. Y. (2008). Effect of rainfall regime and slope on runoff in a gullied loess region on the loess plateau in China. *Environ. Manage.* 42, 402–411. doi: 10.1007/s00267-008-9122-6

Fedoroff, N. V., Battisti, D. S., Beachy, R. N., Cooper, P. J. M., Fischhoff, D. A., Hodges, C. N., et al. (2010). Radically rethinking agriculture for the 21st Century. *Science* 327, 833–834. doi: 10.1126/science.1186834

Fiener, P., and Auerswald, K. (2009). Spatial variability of rainfall on a sub-kilometre scale. *Earth Surf. Process. Landf.* 34, 848–859. doi: 10.1002/esp.1779

Finnerty, B. D., Smith, M. B., Seo, D.-J., Koren, V., and Moglen, G. E. (1997). Space-time scale sensitivity of the Sacramento model to radar-gage precipitation inputs. *J. Hydrol.* 203, 21–38. doi: 10.1016/S0022-1694(97)00083-8

Gaudin, R., Celette, F., and Gary, C. (2010). Contribution of runoff to incomplete off season soil water refilling in a Mediterranean vineyard. *Agric. Water Manag.* 97, 1534–1540. doi: 10.1016/j.agwat.2010.05.007

Hardie, W. J., and Considine, J. A. (1976). Response of grapes to water-deficit stress in particular stages of development. *Am. J. Enol. Vitic.* 27, 55–61.

Hargreaves, G. H., and Samani, Z. A. (1982). Estimating potential evapotranspiration. *J. Irrig. Drain. Div.* 108, 225–230.

Hargreaves, G. H., and Samani, Z. A. (1985). Reference crop evapotranspiration from temperature. *Appl. Eng. Agric.* 1, 96–99. doi: 10.13031/2013.26773

Hatfield, J. L., Sauer, T. J., and Prueger, J. H. (2001). managing soils to achieve greater water use efficiency. *Agron. J.* 93, 271–280. doi: 10.2134/agronj2001.932271x

Hofmann, M., Lux, R., and Schultz, H. R. (2014). Constructing a framework for risk analyses of climate change effects on the water budget of differently sloped vineyards with a numeric simulation using the Monte Carlo method coupled to a water balance model. *Front. Plant Sci.* 5:645. doi: 10.3389/fpls.2014.00645

Howell, T. A. (2001). Enhancing water use efficiency in irrigated agriculture. *Agron. J.* 93, 281–289. doi: 10.2134/agronj2001.932281x

Keesstra, S., Mol, G., De Leeuw, J., Okx, J., Molenaar, C., De Cleen, M., et al. (2018). Soil-related sustainable development goals: four concepts to make land degradation neutrality and restoration work. *Land* 7:133. doi: 10.3390/land7040133

Lebon, E., Dumas, V., Pieri, P., and Schultz, H. R. (2003). Modelling the seasonal dynamics of the soil water balance of vineyards. *Funct. Plant Biol.* 30, 699–710.

Lopes, C. M., Santos, T. P., Monteiro, A., Rodrigues, M. L., Costa, J. M., and Chaves, M. M. (2011). Combining cover cropping with deficit irrigation in a Mediterranean low vigor vineyard. *Sci. Hortic.* 129, 603–612. doi: 10.1016/j.scienta.2011.04.033

Marguerit, E., Brendel, O., Lebon, E., Van Leeuwen, C., and Ollat, N. (2012). Rootstock control of scion transpiration and its acclimation to water deficit are controlled by different genes. *New Phytol.* 194, 416–429. doi: 10.1111/j.1469-8137.2012.04059.x

Oke, T. R. (1987). *Boundary Layer Climates*,2nd Edn. New York, NY: Routledge.

Pauthier, B., Bois, B., Castel, T., and Richard, Y. (2014). Note technique d'implantation d'un réseau de pluviomètres en terrain viticole sur la côte de Beaune (France). *Climatologie* 11, 34–46. doi: 10.4267/climatologie.570

Pellegrino, A., Gozé, E., Lebon, E., and Wery, J. (2006). A model-based diagnosis tool to evaluate the water stress experienced by grapevine in field sites. *Eur. J. Agron.* 25, 49–59. doi: 10.1016/j.eja.2006.03.003

Pieri, P., and Gaudillère, J. P. (2003). Sensitivity to training system parameters and soil surface albedo of solar radiation intercepted by vine rows. *VITIS - J. Grapevine Res.* 42:77.

Pieri, P., and Gaudillere, J.-P. (2005). "Vines water stress derived from a soil water balance model - sensitivity to soil and training system parameters," in *Proceedings of the XIV International GESCO Viticulture Congress*, (Geisenheim: Groupe d'Etude des Systemes de COnduite de la vigne), 457–463.

Quénol, H. (2014). *Changement Climatique et Terroirs Viticoles*. Paris: Lavoisier.

Quénol, H., and Bonnardot, V. (2014). A multi-scale climatic analysis of viticultural terroirs in the context of climate change: the "TERADCLIM" project. *J. Int. Sci. Vigne Vin.* (Special Laccave) 23–32.

Quiquerez, A., Chevigny, E., Allemand, P., Curmi, P., Petit, C., and Grandjean, P. (2014). Assessing the impact of soil surface characteristics on vineyard erosion from very high spatial resolution aerial images (Côte de Beaune, Burgundy, France). *Catena* 116, 163–172. doi: 10.1016/j.catena.2013.12.002

Reynolds, A. G., and Heuvel, J. E. V. (2009). Influence of grapevine training systems on vine growth and fruit composition: a review. *Am. J. Enol. Vitic.* 60, 251–268.

Riou, C., Valancogne, C., and Pieri, P. (1989). Un modele simple d'interception du rayonnement solaire par la vigne. *Verification experimentale. Agronomie* 9, 441–450. doi: 10.1051/agro:19890502

Rockström, J., Karlberg, L., Wani, S. P., Barron, J., Hatibu, N., Oweis, T., et al. (2010). Managing water in rainfed agriculture—The need for a paradigm shift. *Compr. Assess. Water Manag. Agric.* 97, 543–550. doi: 10.1016/j.agwat.2009.09.009

Rodrigo Comino, J., Quiquerez, A., Follain, S., Raclot, D., Le Bissonnais, Y., Casalí, J., et al. (2016). Soil erosion in sloping vineyards assessed by using botanical indicators and sediment collectors in the Ruwer-Mosel valley. *Agric. Ecosyst. Environ.* 233, 158–170. doi: 10.1016/j.agee.2016.09.009

Rodrigo-Comino, J., Keesstra, S., and Cerdà, A. (2018). Soil erosion as an environmental concern in vineyards: the case study of celler del roure, eastern spain, by means of rainfall simulation experiments. *Beverages* 4:31. doi: 10.3390/beverages4020031

Rossato, L., Alvalá, R. C., dos, S., Marengo, J. A., Zeri, M., Cunha, A. P. M., et al. (2017). Impact of soil moisture on crop yields over brazilian semiarid. *Front. Environ. Sci.* 5:73. doi: 10.3389/fenvs.2017.00073

Seguin, G. (1986). "Terroirs" and pedology of vinegrowing. *Experientia* 42, 861–873. doi: 10.1007/bf01941763

Sivakumar, M. V. K., and Hatfield, J. L. (1990). Spatial variability of rainfall at an experimental station in Niger, West Africa. *Theor. Appl. Climatol.* 42, 33–39. doi: 10.1007/BF00865524

van Leeuwen, C. (2010). "9 - Terroir: the effect of the physical environment on vine growth, grape ripening and wine sensory attributes," in *Managing Wine Quality Woodhead Publishing Series in Food Science, Technology and Nutrition*, ed. A. G. Reynolds, (Sawston: Woodhead Publishing), 273–315. doi: 10.1533/9781845699284.3.273

van Leeuwen, C., Tregoat, O., Chone, X., Bois, B., Pernet, D., and Gaudillère, J. P. (2009). Vine water status is a key factor in grapevine ripening and vintage quality for red Bordeaux wine. How can it be assessed for vineyard management process?. *J. Int. Sci. Vigne Vin* 43, 121–134.

Vaudour, E., Costantini, E., Jones, G. V., and Mocali, S. (2015). An overview of the recent approaches to terroir functional modelling, footprinting and zoning. *Soil* 1, 287–312. doi: 10.5194/soil-1-287-2015

Villarini, G., Mandapaka, P. V., Krajewski, W. F., and Moore, R. J. (2008). Rainfall and sampling uncertainties: a rain gauge perspective. *J. Geophys. Res. Atmospheres* 113:D11102. doi: 10.1029/2007JD009214

Wang, H., Zhang, L., Dawes, W. R., and Liu, C. (2001). Improving water use efficiency of irrigated crops in the North China Plain — measurements and modelling. *Agric. Water Manag.* 48, 151–167. doi: 10.1016/S0378-3774(00)00118-9

Ward, F. A., and Michelsen, A. (2002). The economic value of water in agriculture: concepts and policy applications. *Water Policy* 4, 423–446. doi: 10.1016/S1366-7017(02)00039-9

World Meteorological Organization ed. (2008). *Guide to Hydrological Practices*. 6th ed. Geneva, Switzerland: WMO.

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Conservation of $^{87}Sr/^{86}Sr$ During Wine-Making of White Wines: A Geochemical Fingerprint of Geographical Provenance and Quality Production

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The measuring of $^{87}Sr/^{86}Sr$ in wine, grape, and bioavailable soil fraction samples with the same uncertainty of geological materials allows fully comparing the whole wine-production chain with the peculiar geochemical isotope signature of any geographic area. Indeed, this signature is the same as the final product inherited by the soil bioavailable fraction and, in turn, by the geological substratum of the vineyard. On the other hand, the few data available in literature that referred to white wines cast doubts for the use of this geographic tracer due to the common use of geological derived additives, such as bentonite, in the white wine-making procedure, which may overprint the original geochemical signature of the vineyard substratum. To tackle this issue, we analyzed the Sr-isotope compositions of four white wines produced over a period of almost 10 years in a high-quality organic farm, located on the volcanic units of the Vulsini Volcanic District (southern Tuscany, Italy). The $^{87}Sr/^{86}Sr$ values of rock, soil, grape, grape juice, must, and wine were compared among them and further weighted against the isotope fingerprint of the bentonite and yeast employed during the wine-making process. The $^{87}Sr/^{86}Sr$ values from the entire white-wine production chain reveal that no variations are observed from the signature imprinted by the original geological substratum (rocks and soils), suggesting that no further contribution is given by the addition of bentonite and yeast to the white wine Sr-isotope values. On the other hand, intermediate $^{87}Sr/^{86}Sr$ compositions are found when grapes from different vineyards are used for making multi-cultivar wine blends. Indeed, the experimental data clearly show that the Sr isotope composition is maintained through the wine-making process for white as well as for red wines. Both grape and final wine preserved the isotope signature inherited from the labile fraction of the soil where the vines are farmed. Our data thus confirm, also for white wines, the robustness of the Sr-isotope tool in studies where it is important to define *terroirs* and geographic provenance.

Keywords: geologic and geographic traceability, Sr-isotope composition, wine making processes, white wines, wine geochemistry

INTRODUCTION

The adoption of isotopic ratios of heavy radiogenic elements (i.e., Sr and Nd) as possible geochemical tracers of food provenance is gaining scientific consensus within the scientific literature (e.g., Horn et al., 1993; Martin et al., 1999; Roβmann et al., 2000; Barbaste et al., 2002; Kawasaki et al., 2002; Fortunato et al., 2004; Kelly et al., 2005; Voerkelius et al., 2010; Bong et al., 2012; Durante et al., 2013; Marchionni et al., 2013, 2016; Song et al., 2014; Medini et al., 2015; Petrini et al., 2015; Coelho et al., 2017; Tescione et al., 2018; Tommasini et al., 2018).

Preliminary studies using Sr isotopes as possible fingerprint for deciphering the geographic provenance in high quality wines provided questionable results essentially due to the analytical uncertainty of $^{87}Sr/^{86}Sr$ measurements reported, which were usually larger than the Sr-isotopic variability shown by most of the rocks/soils on Earth (e.g., Horn et al., 1993; Almeida and Vasconcelos, 2003, 2004; Vorster et al., 2010; Di Paola-Naranjo et al., 2011). In the last years the mass spectrometry analytic techniques were improved, allowing the researchers to perform Sr isotope measurements on samples of grape, must, wine, and soil with the same analytical precision of that obtained for geologic materials, thus enabling the comparison of the Sr radiogenic isotope composition in red wines to their own terroir of geographic provenance (e.g., Boari et al., 2008; Marchionni et al., 2013, 2016; Tescione et al., 2015; Durante et al., 2016, 2018; Vinciguerra et al., 2016; Braschi et al., 2018; Epova et al., 2019).

On the other hand, among previous studies, the few dealing with white wines missed either to consider the full wine-making production chain (Petrini et al., 2015) or just considered a geographical areas where the isotopic variability was not large enough to discriminate among different substrata (Vorster et al., 2010). The study of Petrini et al. (2015) focused on the comparison between the isotopic composition of the grape components (skin, grape, seeds, and must) used for *Prosecco* white wine and that of the labile fraction in soils (i.e., bioavailable). They observed a statistically significant correspondence between must and soil but the whole isotope range given by the must was so large that encompassed most of the isotopic compositions of the rocks present in the restricted investigated area. Vorster et al. (2010) also employed the Sr isotopes as tracer for provenance on white wine, but their results did not discriminate sufficiently among wines from different regions. In summary, both these studies did not demonstrate the applicability of this tracing technique to white wines and did not solve the question if the use of bentonite may overprint the Sr isotopic signature acquired by the soil and rocks of the substratum of the vineyard.

Recently, Marchionni et al. (2013), during the setting of the analytical protocol for measuring Sr isotope composition on wine samples, analyzed different wines (i.e., red and white wines) from the Italian peninsula each deriving from different and peculiar geologic background (i.e., volcanic and sedimentary). In that study the authors showed that while the isotopic signatures of red wines are all well correlated with respect to their geologic background, a variety of the white wine from Tuscany, the *Ansonico del Giglio*, showed on the contrary a large decoupling in the Sr isotope composition from the geologic substrata of the area of production. The authors suggested that the isotope signature of the *Ansonico del Giglio* wine could be affected by the contribution of other components in addition to those directly inherited from the geologic substratum of the vineyard, such as geologic additives (i.e., bentonite) used during the white wine making procedure.

In this paper we present a detailed experimental study applied of the $^{87}Sr/^{86}Sr$ signature along the whole oenological food-chain from the grape, through the must to the final product, the wine to test the suitability of radiogenic isotopes as tracers for provenance and authenticity also to white wines. In addition, we investigated the possible contribution of bentonite and yeast, used during the wine-making procedure of white wine, to the final $^{87}Sr/^{86}Sr$ of the wine.

To achieve high-detailed results we adopted a sampling strategy relying white wines produced from a single high-quality organic farm, well constrained in time and for the geological and pedological characteristics of the substrata of the vineyard. In addition, the $^{87}Sr/^{86}Sr$ composition of yeast and bentonites were also determined in order to evaluate the possible effect of external components during the white wine-making process.

This study is focused on (i) improving the limited existing database on white wines, (ii) testing the eventual influence on the $^{87}Sr/^{86}Sr$ of the wine of geological and biological additives (i.e., bentonite and yeast) during the different wine-making steps (i.e., clarification and fermentation), and (iii) investigating the applicability of Sr radiogenic isotopes also on blended white wines, where different grape varieties, from different vineyards/parcels, are usually mixed.

MATERIALS AND METHODS

The samples of grape, must, and wine presented in this study are all from one single organic farm and winery located in southern Tuscany, close to the town of Pitigliano, an area set on young volcanic rocks belonging to the Latera volcano succession (e.g., Conticelli et al., 1991, 2010; **Figure 1**, see also **Supplementary Material**). The organic farm and the relative parcels cover a total area of about 72 ha, although divided in two distinct vineyards, about 5 km far from each other, characterized by the presence of different geologic bedrocks, and thus soils, with a different Sr-isotopic composition (e.g., author's unpublished data; Conticelli et al., 2015; Tescione et al., 2018). The largest vineyard of the farm is located in the *Pian de' Conati* area (hereafter in tables and figures indicated as "vineyard *a*"), with soil derived from the *Grotte di Castro* ignimbrite (inset "a" in **Figure 1**). This is the vineyard where most of the white grape varieties (i.e., Greco, Sauvignon and to a minor extent also Trebbiano) are produced. The second vineyard, which was acquired by the farmers later with respect to the beginning of this experimental study, is located in the *Crucignano* area (inset "b" in **Figure 1** and hereafter indicated in figures and tables as "vineyard *b*"). This vineyard is characterized by soil derived from the *Pitigliano Formation* and it is devoted to the growth of the Trebbiano cultivar.

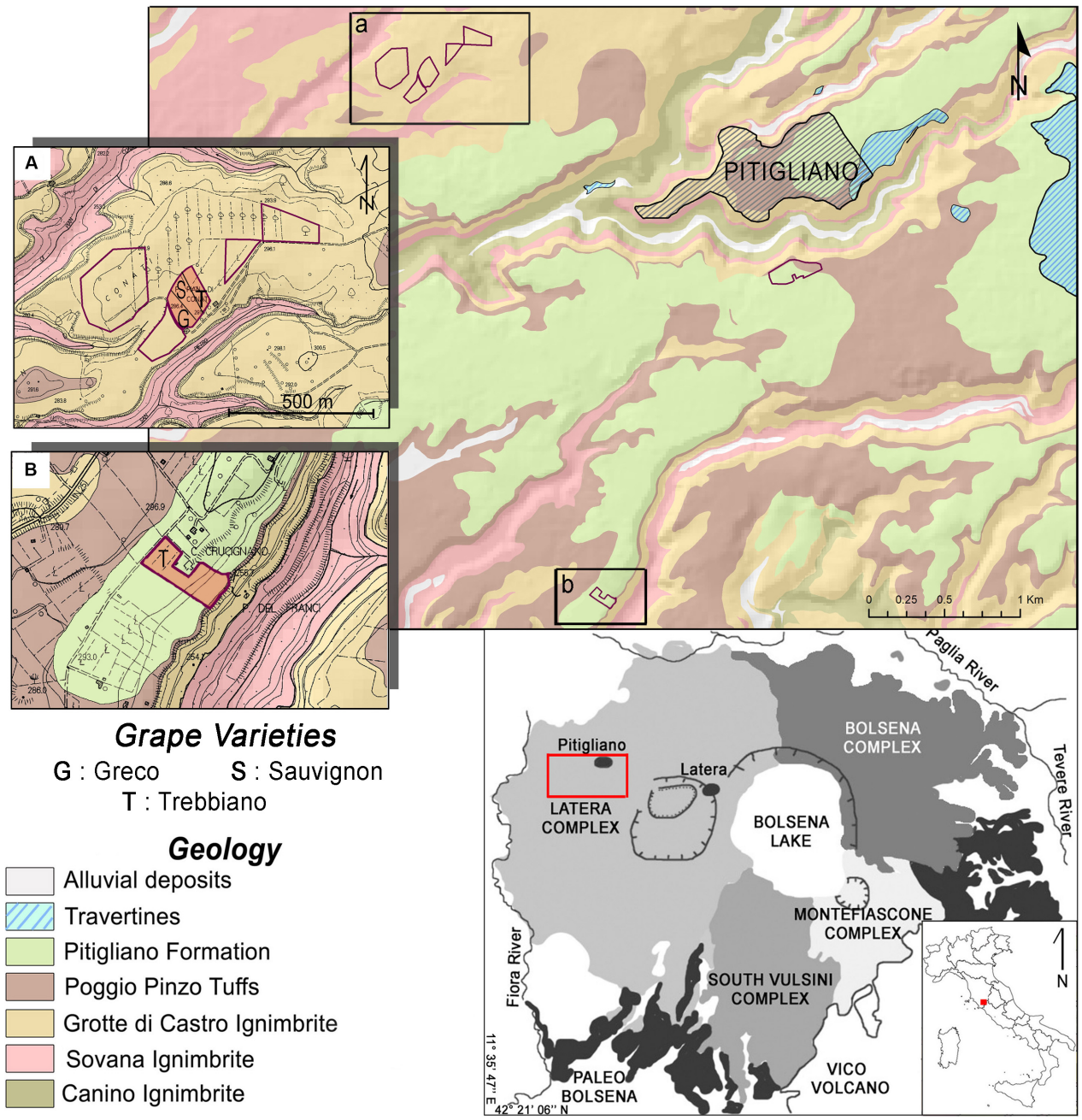

FIGURE 1 | Geological sketch map of the study area (redrawn after Tescione et al., 2018), with rocks belonging to the Latera volcano succession, the last erupting volcano of the Vulsini district (e.g., Conticelli et al., 2010). It is worth noting that the *Pian de' Conati* vineyards fully developed within the Eutric Epileptic Andosols (IUSS, 2006) (autoct. *Aia di Tufi* soil; http://www.lamma.rete.toscana.it/territorio/cartografia-tematica/pedologia/carta-dei-suoli), which derive by the pedogenization of the Grotte di Castro formation (Vezzoli et al., 1987). On the other hand, the *Crucignano* vineyard developed within the Epileptic Andosols (IUSS, 2006) (autoc. *Farmacista* soil), which is derived from the pedogenization of the Pitigliano formation (Vezzoli et al., 1987). See also **Supplementary Material**.

Geological and Pedological Setting and Geochemical Framework

Both vineyards lie on the volcanic succession of the Latera volcano in the surrounding of the Pitigliano village. This volcano belongs to the Vulsini Volcanic District, a 1,500 km^2 widespread volcanic area formed in the time span from 591 to 111 kyr BP (Conticelli et al., 1986, 1987, 2010; Vezzoli et al., 1987; Turbeville, 1992; Marra et al., 2020a). The volcanism produced a thick sequence of pyroclastic deposits and lava flows, erupted from five main volcanic apparata, namely, the Paleo-Bolsena, the Bolsena, the South-Vulsini, the Montefiascone, and the Latera volcanoes (i.e., Vezzoli et al., 1987; Conticelli et al., 2010; Palladino et al.,

2010; Marra et al., 2020b). These coalescent volcanoes, with the exception of South Vulsini one, were characterized by similar eruptive styles with ignimbrite-forming eruptions preceded and followed by effusive and strombolian activities, the latters usually taking place along a peripheral circum-caldera fault system.

The volcanic activity in western sectors of the Vulsini district is dominated by the Latera volcano, which was active from 429 to 145 ka (Vezzoli et al., 1987; Conticelli et al., 2010; Palladino et al., 2010). Lavas were confined in the early and late stage of the volcano, whilst pyroclastic activity occurred between 278 and 166 ka, with the pileup of six main ignimbrites inter-bedded to some events of pyroclastic fall and surges (e.g., Metzeltin and Vezzoli, 1983; Conticelli et al., 1986, 1987, 2010; Nappi et al., 1994). A large polygenic caldera was formed, and several post-caldera lava events were poured out along the rim and within the depression itself with the emplacement of Poggio Pinzi Tuffs, Pitigliano Formation, and final lava flows (e.g., Conticelli et al., 1987, 1991; Nappi et al., 1991; Capaccioni et al., 1997; Capaccioni and Cuccoli, 2005).

The whole composition of Latera volcanic rocks is alkali-potassic with differentiated rocks dominating over mafic ones. Leucite-bearing rocks made up the basal lava plateau and the caldera-forming ignimbrites, but they are also found during the post-caldera activity, erupted coevally with leucite-free rocks (e.g., Varekamp, 1979; Conticelli et al., 1986, 1987, 1991; Turbeville, 1993). Sr-isotopes of Latera rocks range from 0.70933 to 0.71176, with post-caldera leucite-free lavas (0.70991–0.71012) well within the Latera range (Author's unpublished data; Conticelli et al., 1991, 2015).

In a recent paper, Tescione et al. (2018) made a survey of the geological and pedological substrata of the of the *Sassotondo* vineyards. The *Pian de' Conati* vineyard (inset "a" in **Figure 1**) is characterized by a geological substratum made by the Grotte di Castro ignimbrite for a thickness of 15 meters, which gave origin to a Eutric Epileptic Andosols (autoc. *Aia di Tufi* soil). The *Crucignano* vineyard (inset "b" in **Figure 1**) has a geological substratum made by Pitigliano Formation that developed Epileptic Andosols (autoc. *Farmacista* soil).

Sampling

A total amount of 42 samples of grape of each variety (n = 19), grape juice (n = 7), and must (n = 16) were collected from the 2013 to 2016 vintage years during multiple sampling campaigns. In addition, several samples of white wines (n = 30, both mono-cultivar and blended varieties) produced from 2006 to 2015 years have also been collected to investigate the evolution of the isotopic properties over a longer period of time. The grape and juice were sampled before or immediately after the harvesting, musts samples before the filtration using bentonite, and wines before or after the bottling. All the data are original, with the only exception for a few of the grape samples, which are from Tescione et al. (2018). These authors presented a study based on samples from the same farm and investigated the correlation between the isotopic composition of the grape alone (of both red and white varieties) and that of the soil bioavailable fraction. Such correlation was shown to be substantial for all the grape variety with a minor shift for the Sauvignon cultivar, which showed a considerably large range of isotopic composition. The three grape varieties considered in Tescione et al. (2018) were Greco, Sauvignon, and Trebbiano cultivar. The first two belong exclusively to vineyard *a* (*Pian de' Conati*) and covered the vintage years 2013–2016, whereas the latter is mostly from vineyard *b* (*Crucignano*) and covered only the 2015–2016 vintage years.

In this study we reconsidered all the white grape samples of Tescione et al. (2018), re-evaluating the previous measurements and adding new samples in order to check whether the isotopic variability is preserved or not through time (see **Table 1**). Doing that, we expanded the dataset of grape samples including also grape-juice, namely, the immediately subsequent step of grape-crushing before the additive addition. Aiming in particular to investigate the causes of the isotopic variability reported for the Sauvignon cultivar, we improved the dataset with new samples covering the 2013–2015 vintage period, the same period covered in Tescione et al. (2018), plus the 2016 data, which became available at the time of this study. At the same time, we also aimed to verify the consistency of the isotopic signature of the other varieties (i.e., Greco and Trebbiano) through time. Only one sample of grape-juice from the 2013 vintage year was available for Greco. In the case of Trebbiano cultivar, few samples of grape from both areas of production (i.e., *Pian de' Conati* and *Crucignano* areas, respectively) were included along with those of the dataset of Tescione et al. (2018), whereas no sample of grape-juice was available for the same cultivar (see **Table 2**). Must samples cover the 2013–2015 vintage years and are available for the three grape cultivars (**Table 3**).

The selection of analyzed wines concerns both blended and mono-cultivar wines. Two blended wines are the *Numero Sei* wine and the *Isolina* wine. The *Numero Sei* belongs to the *Bianco Maremma Toscana IGT* label and is composed of two grape varieties (Greco and Sauvignon in equal amounts), both grown in the same vineyard of *Pian de' Conati* (vineyard *a*). The *Isolina* belongs to the *Bianco di Pitigliano* DOC and it is a blend of the three grape varieties (Greco, Sauvignon, and Trebbiano) in different percentages (usually about 70% of Trebbiano, 15% of Sauvignon and 10% of Greco, with a minor contribution of other cultivars), which can vary through the vintage years according to the productivity of the single varieties. While the Greco and the Sauvignon grapes are exclusively grown in the *Pian de' Conati* area (vineyard *a*), the Trebbiano cultivar is grown in both areas, but most of the grapes produced in the last vintage years are from the *Crucignano* area (vineyard *b*).

Two mono-cultivar wine varieties are also included in this study, the *Greco* and *Tufo Bianco* wines of the 2011 and 2015 vintage year, respectively. The first is entirely composed by Greco grapes growing exclusively on vineyard *a*. The latter, for the vintage year of 2015, is a mono-cultivar wine of the Trebbiano grape variety produced by grapes harvested from the vineyard *b*.

In addition to the raw materials for wine production, two samples of natural additives (i.e., bentonite and yeast) usually employed by the farmers were included in the study in order to explore their potential contribution on the Sr isotope composition of the produced white wines. Bentonite is a type

TABLE 1 | Isotopic composition of the analyzed wines from the *Sassotondo* winery divided by vintage years and grape variety, area of provenance.

Year	Vineyard	Grape variety	Wine name	$^{87}Sr/^{86}Sr_m$	2 se	*n*
2011	a	Greco	*Greco*	0.710053	±0.000006	114
2006	a	Greco, Sauvignon	*Numero Sei*	0.709983	±0.000006	115
2006	a	Greco, Sauvignon	*Numero Sei*	0.709972	±0.000006	116
2006	a	Greco, Sauvignon	*Numero Sei*	0.709977	±0.000008	116
2007	a	Greco, Sauvignon	*Numero Sei*	0.709988	±0.000006	116
2007	a	Greco, Sauvignon	*Numero Sei*	0.709984	±0.000006	114
2007	a	Greco, Sauvignon	*Numero Sei*	0.709981	±0.000007	115
2011	a	Greco, Sauvignon	*Numero Sei*	0.709970	±0.000007	115
2011	a	Greco, Sauvignon	*Numero Sei*	0.709978	±0.000007	116
2011	a	Greco, Sauvignon	*Numero Sei*	0.709975	±0.000007	115
2015	b	Trebbiano	*Tufo Bianco*	0.709567	±0.000009	116
2015	b	Trebbiano	*Tufo Bianco*	0.709565	±0.000006	117
2006	a	Greco, Sauvignon, Trebbiano	*Isolina*	0.710014	±0.000006	116
2006	a	Greco, Sauvignon, Trebbiano	*Isolina*	0.710019	±0.000006	115
2006	a	Greco, Sauvignon, Trebbiano	*Isolina*	0.710022	±0.000007	115
2007	a	Greco, Sauvignon, Trebbiano	*Isolina*	0.709927	±0.000007	115
2007	a	Greco, Sauvignon, Trebbiano	*Isolina*	0.709928	±0.000007	118
2011	a, b	Greco, Sauvignon, Trebbiano	*Isolina*	0.709691	±0.000007	115
2011	a, b	Greco, Sauvignon, Trebbiano	*Isolina*	0.709677	±0.000006	114
2011	a, b	Greco, Sauvignon, Trebbiano	*Isolina*	0.709693	±0.000006	116
2012	a, b	Greco, Sauvignon, Trebbiano	*Isolina*	0.709688	±0.000006	114
2013	a, b	Greco, Sauvignon, Trebbiano	*Isolina*	0.709517	±0.000007	114
2013	a, b	Greco, Sauvignon, Trebbiano	*Isolina*	0.709510	±0.000006	111
2014	a, b	Greco, Sauvignon, Trebbiano	*Isolina*	0.709853	±0.000006	116
2014	a, b	Greco, Sauvignon, Trebbiano	*Isolina*	0.709848	±0.000007	116
2014	a, b	Greco, Sauvignon, Trebbiano	*Isolina*	0.709882	±0.000009	115
2015	a, b	Greco, Sauvignon, Trebbiano	*Isolina*	0.709343	±0.000006	113
2015	a, b	Greco, Sauvignon, Trebbiano	*Isolina*	0.709351	±0.000007	116
2015	a, b	Greco, Sauvignon, Trebbiano	*Isolina*	0.709589	±0.000007	114
2015	a, b	Greco, Sauvignon, Trebbiano	*Isolina*	0.709591	±0.000006	114

The mean values of the (n) measurements and 2 se deviations are reported. The Greco and the Tufo Bianco are mono-cultivar white wines. The Numero Sei is a blend composed by 50% of Greco and 50% Sauvignon. The Isolina wine is a blend composed by ca. 70% of Trebbiano, 15% of Sauvignon and 10% of Greco, with a minor contribution of other cultivars; these percentages can vary through the vintage years according with productivity of the single varieties.

of montmorillonite clay widely employed as a fining agent for clarification. Together with other agents, such as tannins and casein, bentonite can speed the settling of particulate matter, induce partial decolorization, and correct for the addition of excessive amounts of proteinaceous nutrients (i.e., amino acids) inducing their precipitation (Jackson, 2008). Given that bentonite settles very quickly, and it is also easily filtered, it does not represent itself with neither stability nor clarification problems. In comparison with other fining agents it is also considered to have a minimal effect on the sensory properties of the final wine. In this light we want to test if the addition of bentonite could produce any effect of the radiogenic Sr isotope composition of the wine (Horn et al., 1993; Durante et al., 2016). The yeasts are added directly into the grape juice or into the must to encourage the process of fermentation, promoting the conversion of the sugar contained into ethanol.

Aiming to explore the potential contribution of such additives to the white wines, we selected the bentonite and the yeast samples to analyze directly from the same batch of additives the farmers employed during their wine-production.

In all the figures shown in this study, the range of variability of the labile fraction from the soil is always reported, both for the *Pian de' Conati* and for the *Crucignano* areas (vineyard *a* and *b*, respectively). These values are the same experimentally measured following the procedure of Marchionni et al. (2016) and published by Tescione et al. (2018), which discussed in detail the isotopic signature of bedrock, soil, and bio-available fraction from the soil.

Sample Preparation and Analysis

Sample preparation and measurements were performed following the procedure described in Marchionni et al. (2016) and Tescione et al. (2018) and specifically adopted for the treatment of samples such as grape, grape-juice, soil, and bio-available fraction from the soil. The Sr isotope composition was measured through Thermal Ionization Mass Spectrometry (TIMS) on a Triton T1 (Thermo Finnigan) at the Earth Science Department of the Università degli Studi di Firenze. Before isotopic determination, the Sr elemental concentration of bentonite and yeast was analyzed through ICP-MS at the

TABLE 2 | Isotopic composition of the grapes and grape-juices divided by vintage years and grape variety.

Year	Vineyard	Grape variety	$^{87}Sr/^{86}Sr_m$	2 se	*n*
2013	a	Greco grape	0.709749	±0.000010	55
2013	a	Greco grape-juice	0.710040	±0.000007	113
2014	a	Greco grape	0.709960	±0.000006	112
2014	a	Greco grape	0.710029	±0.000007	117
2015	a	Greco grape	0.710082	±0.000008	116
2015	a	Greco grape	0.710050	±0.000007	115
2016	a	Greco grape	0.710129	±0.000048	101
2016	a	Greco grape	0.710005	±0.000086	101
2013	a	Sauvignon grape	0.709738	±0.000010	75
2013	a	Sauvignon grape-juice	0.709939	±0.000008	114
2014	a	Sauvignon grape	0.709749	±0.000010	55
2014	a	Sauvignon grape	0.710048	±0.000007	116
2014	a	Sauvignon grape-juice	0.710059	±0.000006	113
2014	a	Sauvignon grape-juice	0.710050	±0.000006	111
2014	a	Sauvignon grape-juice	0.710052	±0.000007	115
2015	a	Sauvignon grape	0.710095	±0.000042	60
2015	a	Sauvignon grape	0.710065	±0.000008	81
2016	a	Sauvignon grape	0.710042	±0.000028	82
2016	a	Sauvignon grape-juice	0.710110	±0.000007	116
2016	a	Sauvignon grape-juice	0.710105	±0.000007	116
2015	a	Trebbiano	0.710069	±0.000008	115
2015	a	Trebbiano	0.710049	±0.000008	114
2016	a	Trebbiano	0.710086	±0.000008	92
2016	a	Trebbiano	0.710084	±0.000009	93
2016	b	Trebbiano	0.709527	±0.000007	115
2016	b	Trebbiano	0.709526	±0.000007	114

The mean values of the (n) measurements and 2 se deviations are reported.

TABLE 3 | Isotopic composition of the must divided by vintage years and grape variety.

Year	Grape variety	$^{87}Sr/^{86}Sr_m$	2 se	*n*
2013	Greco	0.710026	±0.000006	113
2014	Greco	0.710021	±0.000006	114
2014	Greco	0.710023	±0.000007	118
2014	Greco	0.710019	±0.000007	114
2015	Greco	0.710048	±0.000007	114
2015	Greco	0.710044	±0.000007	118
2013	Sauvignon	0.709565	±0.000006	114
2015	Sauvignon	0.710050	±0.000007	115
2015	Sauvignon	0.710065	±0.000007	115
2015	Sauvignon	0.710065	±0.000006	113
2013	Trebbiano	0.709415	±0.000008	117
2014	Trebbiano	0.709635	±0.000006	115
2014	Trebbiano	0.709632	±0.000008	116
2014	Trebbiano	0.709643	±0.000006	116
2015	Trebbiano	0.709548	±0.000006	114
2015	Trebbiano	0.709540	±0.000007	114

The mean values of the (n) measurements and 2 se deviations are reported.

University of Bristol (United Kingdom). The sampling strategy and the whole preparation treatment of the grape, grape juice, must, and wines were performed following the procedure reported in Marchionni et al. (2013, 2016). Grape-juice samples were obtained from the collected grapes directly in the laboratory. Must and wines were collected directly at the winery. The grape was rinsed several times with Milli-Q® water and then crushed with skin and seeds. The solution was filtered, and the juice was treated as must and wine samples. About 5 ml of each sample was evaporated and digested first in 3 ml of H_2O_2 (UpA) and then in 2 ml of HNO_3. The whole digestion procedure was repeated twice. The Sr purification was ensured through chromatographic separation using Eichrom Sr-Spec resin (150 μl). Additives were treated following the procedure for geologic samples described in Tescione et al. (2018) for the soil and the bedrock analyses.

The isotope measurements were performed loading some 100–150 ng of sample onto single Re-filament as nitrate form with $TaCl_5$ and H_3PO_4 as activator and to keep the signal stable during the measurements. The Sr isotope ratios were measured in multi-dynamic mode, applying the triple jump procedure (Thirlwall, 1991) described in detail by Avanzinelli et al. (2005). Each reported isotope ratio is the result of 120 cycles (with each cycle representing the average of three measurements performed during the triple-jumping), taken in six blocks, each consisting of 20 cycles with 8 s integration time. An idle time of 3 s was set before the start of the collection after each jump, to eliminate possible memory effect due to the decay of the signal in the Faraday cups (Avanzinelli et al., 2005). The instrumental mass-bias was corrected off-line using the $^{88}Sr/^{86}Sr$ ratio measured on the main configuration (jump 2). The measured and the natural $^{88}Sr/^{86}Sr$ ($^{88}Sr/^{86}Sr_N$ = 8.375209) were used both to calculate the mass discrimination factor (ε) and to subsequently apply the correction through the exponential fractionation law. The $^{87}Sr/^{86}Sr_{triple}$ average value for the NIST SRM987 international reference standard was 0.710248 ± 0.000016 (2σ, *n* = 121).

The reproducibility of the analytical method we used in this study is reported in Marchionni et al. (2013), where 31 different aliquots of the same sample of wine were processed and measured for $^{87}Sr/^{86}Sr$ composition, yielding a 2σ = ±0.000017 (i.e., ±23 ppm), which is well consistent with that of the international reference standard.

RESULTS AND DISCUSSION

The values of $^{87}Sr/^{86}Sr$ of wine, grape, grape-juice, and must are reported in **Tables 1–3**. The isotope compositions of each analyzed matrix are graphically represented (**Figures 2–7**) by box-plot diagrams, where the box is delimited by the 25th and the 75th percentiles of each population, the whiskers show the 10th and the 90th percentiles, whilst the dots represent the outliers at the 5th and 95th percentiles.

The isotope composition of the soil bio-available fractions used as reference in this study are $^{87}Sr/^{86}Sr$ = 0.710107 ± 0.000005 ($2\sigma_m$) for the Pian de' Conati area and $^{87}Sr/^{86}Sr$ = 0.709644 ± 0.000022 for the Crucignano area (averaged values from the values of Tescione et al., 2018).

Figure 2 shows the isotope composition of wines produced both in vineyards *a* and *b* over the 2006–2015 period. Although the record is not complete (e.g., there is a gap from 2008

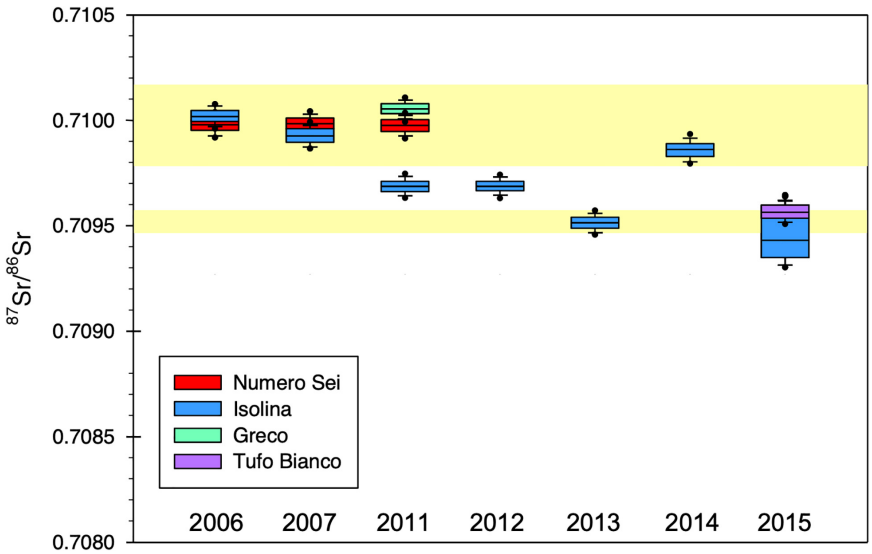

FIGURE 2 | $^{87}Sr/^{86}Sr$ values in the wine samples from different vintage years. Both multiple-variety (*Numero Sei* and *Isolina*) and varietal (*Greco* and *Tufo Bianco*) wines are reported. The box is delimited by the 25th and the 75th percentiles of each population, the whiskers show the 10th and the 90th percentiles, whilst the dots represent the outliers at the 5th and 95th percentiles. The yellow fields represent the variability range (at the 5th and 95th percentiles) of the grapes grown in the two respective areas of *Pian de' Conati* (vineyard *a*) and *Crucignano* (vineyard *b*).

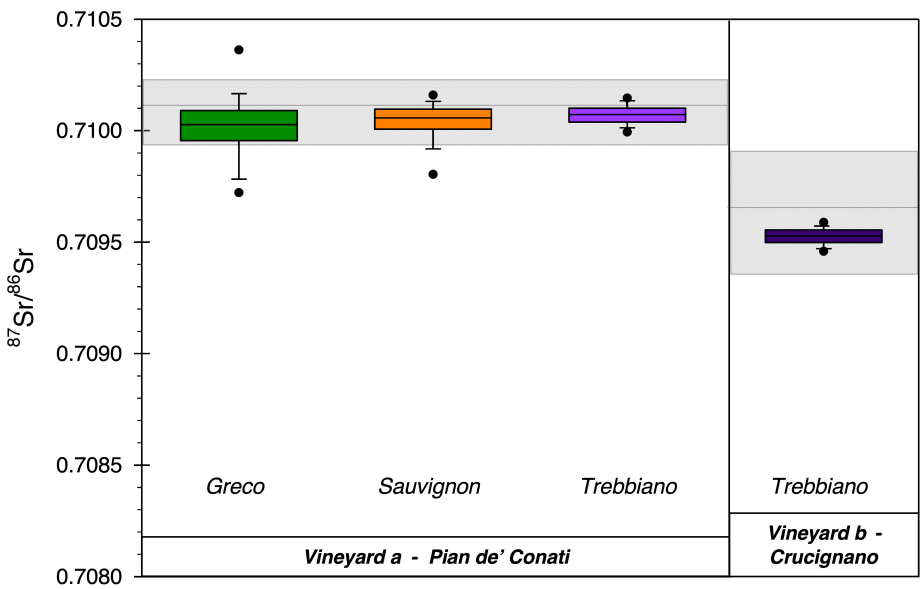

FIGURE 3 | $^{87}Sr/^{86}Sr$ values in grape samples from the different varieties grown on the two different vineyards, which are characterized by distinct geological backgrounds (see more details through the text). The *Pian de' Conati* area (vineyard *a*) is the place where the Greco, Sauvignon and, to minor extent Trebbiano cultivar, are grown. The area of *Crucignano* (vineyard *b*) is committed to Trebbiano cultivar. The box plot features are reported through text and in the caption of **Figure 2**. The gray pattern represents the whole range of variability of the bio-available fraction extracted from the soil of the two respective areas, the solid line represents the mean value.

to 2010) it is possible to highlight several interesting aspects. The box plots refer to the average of the wine of the same type, produced in each year. The yellow fields represent the range of isotopic variability shown by the grape samples from the two different vineyards (see also **Figure 3** and **Table 2**). The comparison between the isotopic composition of wine and grape reinforces the first important assumption that the isotopic signature of wine is preserved through time and is in agreement with that of the grape from which the wine derived. This is needed in particular for the wine samples from vintage years 2006–2013 that do not have any analog grape sample to be compared to.

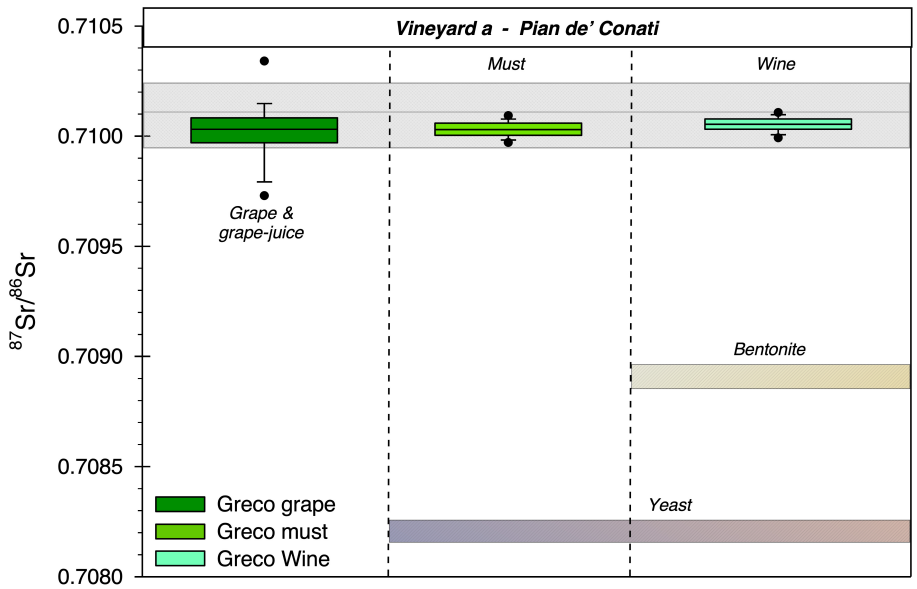

FIGURE 4 | $^{87}Sr/^{86}Sr$ values in grape, juice, musts, and wine for the Greco wine (from the 2011 vintage year). Greco is high-quality single-variety wine, composedby Greco alone, which is growth in the area of *Pian de' Conati* (vineyard *a*). The box plot features are reported through text and in the caption of **Figure 2**. The gray pattern represents the whole range of variability of the soil bioavailable fraction, the solid line represents the mean value. The shaded bars represent the isotopic range of the analyzed additives, bentonite, and yeast, respectively. The length of the bars depends on the wine-making step in which such additives are introduced, thus the different matrix in which we investigated their potential effect.

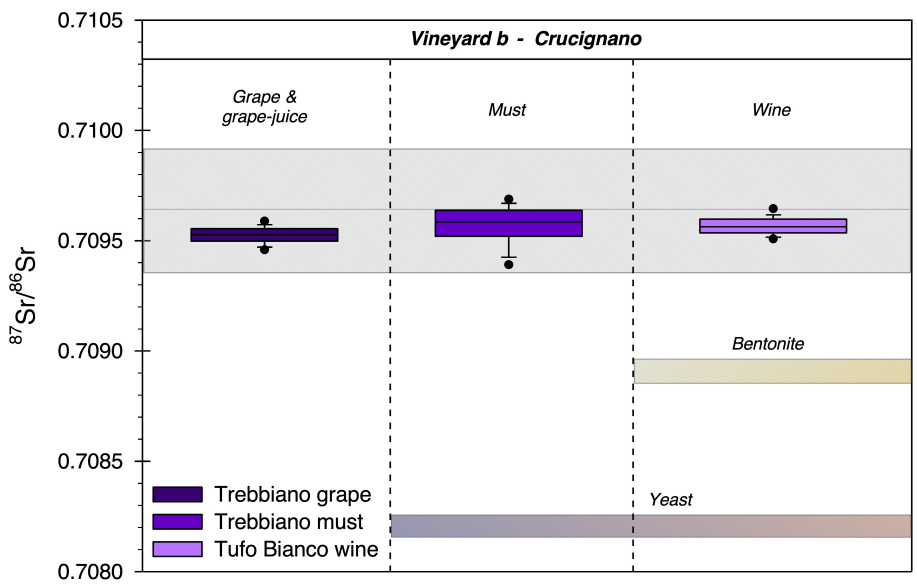

FIGURE 5 | $^{87}Sr/^{86}Sr$ values in grape, juice, musts, and wine for the Tufo Bianco wine (from the 2015 vintage year). Tufo Bianco is high-quality varietal wine,composed by Trebbiano alone, which was harvested in 2015 from the area of *Crucignano* (vineyard *b*). The box plot features are reported through text and in thecaption of **Figure 2**. The gray pattern represents the whole range of variability of the soil bioavailable fraction; the solid line represents the mean value. The shadedbars represent the isotopic range of the analyzed additives, bentonite and yeast, respectively. The length of the bars depends on the wine-making step in which suchadditives are introduced, thus the different matrix in which we investigated their potential effect.

A first important observation concerns the isotope compositions of *Numero Sei* (blended variety) and *Greco* (mono-cultivar) white wine, which are very close to each other, and both fall well within the field of the grapes grown in the vineyard *a*, whereas the mono-cultivar *Tufo Bianco* wine plots are closer to the field of the grapes produced in the vineyard *b*. On the other hand, the *Isolina* wines show variable isotopic composition through the years. Indeed, the

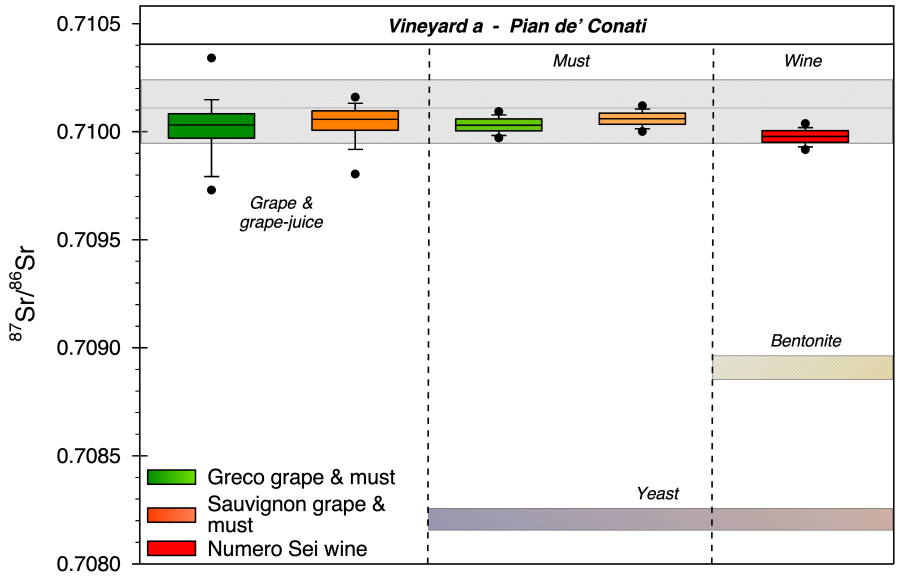

FIGURE 6 | $^{87}Sr/^{86}Sr$ values in grape, juice, musts, and wine for the *Numero Sei* wine (from the 2006, 2007, and 2011 vintage years). *Numero Sei* wine is composed by a blending of Greco and Sauvignon varieties, which are both grown in the area of *Pian de' Conati* (vineyard *a*). The box plot features are reported through text and in the caption of **Figure 2**. The gray pattern represents the whole range of variability of the soil bioavailable fraction; the solid line represents the mean value. The shaded bars represent the isotopic range of the analyzed additives, bentonite, and yeast, respectively. The length of the bars depends on the wine-making step in which such additives are introduced, thus the different matrix in which we investigated their potential effect.

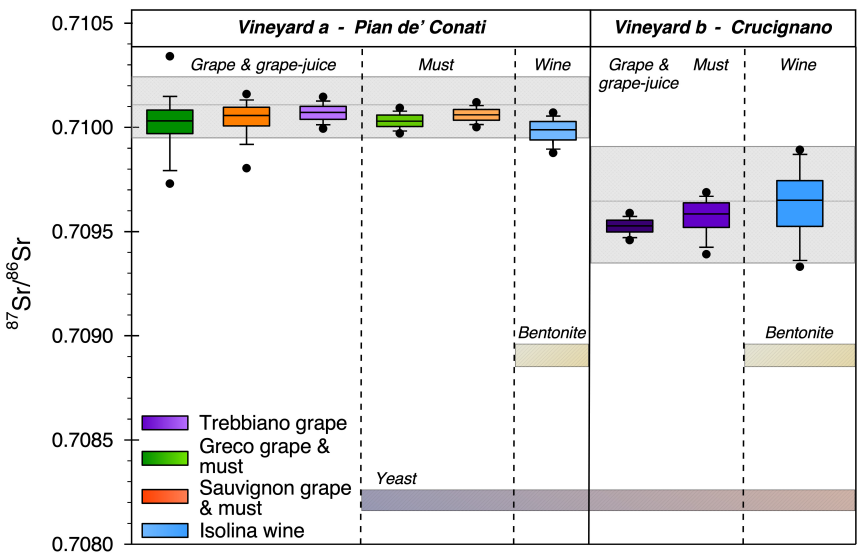

FIGURE 7 | $^{87}Sr/^{86}Sr$ values in grape, juice, musts, and wine for the *Isolina* wine (from the 2006 to the 2015 vintage years). *Isolina* wine is composed by a blending of Greco, Sauvignon, and Trebbiano varieties. Among these grape varieties, Greco and Sauvignon are exclusively grown in the area of *Pian de' Conati* (vineyard *a*), whereas the Trebbiano derive mostly from the *Crucignano* area (vineyard *b*). The final value of the wine depends on the percentages of the three musts employed year by year and on the geologic provenance of the soil on which the grape grew. The box plot features are reported through text and in the caption of **Figure 2**. The gray patterns represent the whole range of variability of the soil bioavailable fraction, respectively, in the *Pian de' Conati* (*a*) and the *Crucignano* (*b*) area. The shaded bars represent the isotopic range of the analyzed additives, bentonite, and yeast, respectively. The length of the bars depends on the wine-making step in which such additives are introduced, thus the different matrix in which we investigated their potential effect.

2006 and 2007 vintage years plot closer to the reference field of grape from the vineyard *a*, whereas, from 2011 to 2015, the isotopic signature becomes more variable and it is generally more consistent with the range of isotopic composition of grapes from vineyard *b*, with the only exception of 2014 vintage year. The different magnitude of the isotopic range of the grapes from vineyard *b*, which is considerably smaller compared to that of vineyard *a*, is clearly presented by the grape box plots

in **Figure 3** and is likely due to the limited smaller number of samples available, compared to those from vineyard *a*. In **Figure 3**, and in all the following figures, the isotope composition of each grape variety is reported and correlated to the soil bio-available fraction (and thus the geographical provenance of the grape) (**Table 2**).

It is clear that all the $^{87}Sr/^{86}Sr$ values of Greco, Sauvignon, and Trebbiano grapes sampled in the *Pian de' Conati* area (vineyard *a*) are consistent with the isotopic signature of the soil bio-available fraction in the same area, as well as the Trebbiano grape samples from the *Crucignano* area (vineyard *b*) that fall well within the field of the respective soil labile fraction (**Figure 3**). This evidence corroborates the fact that the $^{87}Sr/^{86}Sr$ isotope composition is not dependent on the grape variety but on the soil of origin, as already suggested by previous studies (e.g., Marchionni et al., 2016; Braschi et al., 2018; Tescione et al., 2018). The larger variability in $^{87}Sr/^{86}Sr$ values of Greco and Sauvignon grapes with respect to those of Trebbiano (which are harvested from both the two vineyards) is likely related to the time span covered by the grape sampling. Indeed, the first two varieties were sampled over a period of four vintage years (from 2013 to 2016) possibly reflecting small-scale local variations, whereas the Trebbiano variety (in both vineyards) was sampled only during the vintage years 2015–2016.

In **Figure 2**, we showed the presence of a general correlation between wines and grapes, despite that some variabilities in the isotope composition of the two matrices are observed.

The preservation of the isotopic signature from the grape to the wine and the possible occurrence of modifications through the food chain are tested through each step of the production of both mono-cultivar and blended wines, before and after the addition of yeast and bentonite. The yeast is usually added to the grape juice to promote the fermentation of must, and the bentonite is added to the must to improve the effect of filtration before the bottling. Given that the two additives show well distinct isotope signature, we expect to observe, if any, a peculiar contribution of the yeast in the must and wine fractions, while the bentonite is supposed to contribute to the latter fraction only. The isotopic composition of must and additives measured in this study is given in **Tables 3**, **4**.

Greco is a high-quality mono-cultivar white wine produced with the homonymous grape variety, which is derived uniquely from vineyard *a* (**Figure 3**). The comparison reported in **Figure 4** among the $^{87}Sr/^{86}Sr$ values measured on each component of the wine-making procedure demonstrates that although the grape alone shows the more variable range of isotope composition compared to that of must samples and wine, they all substantially overlap well within the error. Moreover, neither must nor wine samples are affected by the addition of yeast or bentonite, respectively, and at the same time, together with the grape-juices, fall well within the field of the bio-available fraction from the vineyard *a* (**Figure 4**).

The same observations are made for the *Tufo Bianco* white wine, exclusively produced with the Trebbiano grapes grown in the vineyard *b* (**Figure 3**). In this case the samples of must show more variable $^{87}Sr/^{86}Sr$ values than grape-juice and wine, but as in the case of *Greco* wine, a perfect overlapping can be observed among the three matrices (**Figure 5**). The absence of any drift toward the bentonite and yeast again suggests that such components do not affect the final isotopic composition of the wine. These evidences demonstrate that the Sr isotope composition of mono-cultivar wines does not change during the different steps of the wine-making procedure, and instead it is strongly dependent on the primary features of the soil where the grape grew.

A similar evidence is achieved in the case of wines made by blending of different grape varieties, which have the same provenance. *Numero Sei* is a high-quality white wine made of a blending of Greco and Sauvignon grapes in equal proportions (**Figure 6**). The isotope signature of the two varieties is similar within the error, indeed they derived from the same vineyard *a*. The measured $^{87}Sr/^{86}Sr$ values of the grapes are preserved through the must to the wine, with negligible effect of the additives. The slight variability of the wine samples can be possibly ascribed to small changes in the proportion of Greco used through the vintage years.

One of the more interesting characteristics shown in **Figure 2** is the extremely variable isotope composition shown by the *Isolina* wine through the vintage years. The temporal distribution of Sr isotopes provides important evidence, highlighting that between the 2006 and 2007 harvest years, the *Isolina* wine, although slightly different form each other, still overlap within the range of variability, whereas after the 2011 vintage year, the $^{87}Sr/^{86}Sr$ composition become less radiogenic and more scattered in absolute value. Considering that the effect of additives can be neglected for both Greco and Trebbiano mono-cultivar and blended wines, the same holds true for the *Isolina* blend (**Figure 7**), suggesting definitely that the addition of bentonite and yeast does not affect the Sr isotope composition of the white wine. The observed variability should be thus ascribed to other reasons.

Following this rationale, if we use the 2011 as a temporal divide, we can observe that the Sr isotope signature of the 2006–2007 wines well fits with that of the Trebbiano grapes sampled in vineyard *a* along with Greco and Sauvignon grapes, and the respective musts. Due to a lack of sample availability, there is no reference value for the must of Trebbiano from vineyard *a*. On the contrary, we measured both grape and must fractions from vineyard *b*, which nicely correlate with the bio-available fraction from the soil of this area (**Figures 5**, **7**). As already observed in the case of the *Tufo Bianco*, the Sr isotope signature is maintained from the grape to the must and, once compared to *Isolina* wine production after the 2011 vintage year, well correlates with that of the wine. The fact that the isotopic composition of both grape juice and must from vineyard *b* covers a range of values that is significantly smaller than that of the 2011–2015 wine requires another process to be considered.

TABLE 4 | Isotopic composition and Sr content of additives.

Additives	Sr ppm	$^{87}Sr/^{86}Sr_m$	2 se	*n*
Bentonite	59.3	0.708910	±0.000007	115
Yeast	11.5	0.708211	±0.000006	116

Such variability could be likely explained if we consider that the proportions of Trebbiano, Greco, and Sauvignon varieties in the *Isolina* blend can vary according to the availability of each grape variety through the vintage years and to the desired characteristics of the final product. This is also not surprising considering that the *Isolina* wine, with respect to the other high-quality analyzed wines, is the variety addressed to the larger distribution chain. In other words, even if within the limits of the DOC label regulation, the proportion of the different grapes in the blend is less rigorous and more depending on the production rate of each variety year by year. Accordingly, when the winery started the production of 2006 harvest year, only the grapes from the vineyard of *Pian de' Conati* were used. On the other hand, grapes from *Crucignano* area (vineyard *b*) were harvested and thus used in the wine-making production only after the 2011 harvest year. Since then, the *Crucignano* vineyard was dedicated to the exclusive production of the Trebbiano mono-cultivar wine, which was used for both *Tufo Bianco* mono-cultivar production and for the blended *Isolina* wine. Thus, the larger isotopic variability of the *Isolina* wine in the period 2011–2015, driven mainly by the samples from 2014 harvest year, can be explained (i) by the use of the more radiogenic Greco and Sauvignon grape variety in a larger proportion than the usual, or (ii) by the employment of grape of the less radiogenic Trebbiano variety from vineyard *b* with minor additions of the more radiogenic Trebbiano grape from the vineyard *a*.

CONCLUSION

In this paper we presented new and original data covering all the steps of the wine-making process, from the grape to the final bottle, applied to a small, organic, high-quality farm over a period of time of almost 10 years. It is the first time that such interval was investigated for the verification of the conservation of the Sr isotopic ratio.

This study demonstrated that the different steps of the wine-making process do not affect the final isotopic composition of the wine. The isotopic signature is instead directly correlated to that of the vineyard pedogenetic substratum and it is transferred through the labile fraction from the soil, through the roots of the plant, to the grapes. Grape-juice and must still preserve the original isotopic composition and are not affected by the addition of materials with different isotopic signature. Neither bentonite nor yeast, added during the wine-making process, contribute in modifying the Sr isotope composition of the wine. All the values related to the different steps of the winemaking process show a strict relationship with the bioavailable fraction of soil, and then the geologic bedrock of the vineyard of origin.

Our results show that the Sr isotope composition not only does not change during the whole productive process but also does not relevantly change through different vintage years, as demonstrated by the correspondence between the isotope signature of wines and grape/must from different vintage years. Indeed, the isotopic tool can be suitably used to confirm the geographic origin of white wines, as it was already demonstrated for red wines. Particular care has to be considered in the case of blending of varieties grown on isotopically heterogeneous geologic bedrocks. This is a quite frequent case because wines are not always only mono-varietal, and often the wineries employ different vineyards that can grow on different geologic bedrocks. For these reasons, the final isotopic ratio in wines could be influenced by the quantity, the percentage, and the respective $^{87}Sr/^{86}Sr$ of each component mixed in the final product, especially if they come from different areas of origin. The possibility to examine all the components of the system could, however, guarantee the product traceability, as well as help to define the *terroir* of provenance for high-quality label.

This study contributes in the assessment of the geographical origin of grapes and wines as relevant for the protection of the labeled products with a certification of origin. At the same time, it provides useful evidences for tracing the geographic provenance of its components.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

SC and MM designed the research. IT, SM, MM, and SC collected the samples and performed the geological survey of the studied areas. SM, IT, MC, and EB made the analytical work. MC and IT wrote the manuscript. All the authors discussed the data, revised, and approved the final form of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2020.00153/full#supplementary-material

REFERENCES

Almeida, C. M. R., and Vasconcelos, M. T. S. (2003). Multielement composition of wines and their precursors including provenance soil and their potentialities as fingerprints of wine origin. *J. Agr. Food Chem.* 51, 4788–4798. doi: 10.1021/jf034145b

Almeida, C. M. R., and Vasconcelos, M. T. S. (2004). Does the winemaking process influence the wine 87Sr/86Sr? *A case study. Food Chem.* 85, 7–12. doi: 10.1016/j.foodchem.2003.05.003

Avanzinelli, R., Boari, E., Conticelli, S., Francalanci, L., Guarnieri, L., Perini, G., et al. (2005). High precision Sr, Nd, and Pb isotopic analyses using the new generation thermal ionisation mass spectrometer ThermoFinnigan Triton-Ti®. *Period. Mineral.* 74, 147–166.

Barbaste, M., Robinson, K., Guilfoyle, S., Medina, B., and Lobinski, R. (2002). Precise determination of the strontium isotope ratios in wine by inductively coupled plasma sector field multicollector mass spectrometry (ICP-SF-MC-MS). *J. Anal. At. Spectrom.* 17, 135–137. doi: 10.1039/b109559p

Boari, E., Tommasini, S., Mercurio, M., Morra, V., Mattei, M., Mulinacci, N., et al. (2008). "87Sr/86Sr of some central and southern Italian wines and its use as fingerprints for geographical provenance," in *OIV 2008 – 31st World Congress of Vine and Wine*, (CD-ROM).

Bong, Y.-S., Shin, W.-J., Gautam, M. K., Jeong, Y.-J., Lee, A.-R., Jang, C.-S., et al. (2012). Determining the geographical origin of Chinese cabbages using multielement composition and strontium isotope ratio analyses. *Food Chem.* 135, 2666–2674. doi: 10.1016/j.foodchem.2012.07.045

Braschi, E., Marchionni, S., Priori, S., Casalini, M., Tommasini, S., Natarelli, L., et al. (2018). Tracing the 87Sr/86Sr from rocks and soils to vine and wine: An experimental study on geologic and pedologic characterisation of vineyards using radiogenic isotope of heavy elements. *Sci. Total Environ.* 628, 1317–1327. doi: 10.1016/j.scitotenv.2018.02.069

Capaccioni, B., and Cuccoli, F. (2005). Spatter and welded air fall deposits generated by fire-fountaining eruptions: cooling of pyroclasts during transport and deposition. *J. Volcanol. Geoth. Res.* 145, 263–280. doi: 10.1016/j.jvolgeores.2005.02.001

Capaccioni, B., Valentini, L., Rocchi, M. B., Nappi, G., and Sarocchi, F. (1997). Image analysis and circular statistics for shape-fabric analysis: applications to lithified ignimbrites. *Bull. Volcanol.* 58, 501–514. doi: 10.1007/s004450050158

Coelho, I., Castanheira, I., Bordado, J. M., Donard, O., and Silva, J. A. L. (2017). Recent developments and trends in the application of strontium and its isotopes in biological related fields. *TrAC-Trend. Anal. Chem.* 90, 45–61. doi: 10.1016/j.trac.2017.02.005

Conticelli, S., Boari, E., Burlamacchi, L., Cifelli, F., Moscardi, F., Laurenzi, M. A., et al. (2015). Geochemistry and Sr-Nd-Pb isotopes of Monte Amiata Volcano, Central Italy: evidence for magma mixing between high-K calc-alkaline and leucititic mantle-derived magmas. *Ital. J. Geosci.* 134, 266–290. doi: 10.3301/ijg.2015.12

Conticelli, S., Francalanci, L., Manetti, P., and Peccerillo, A. (1987). Evolution of Latera Volcano, Vulsinian district (Central Italy): stratigraphical and petrological data. *Period. Mineral.* 56, 175–199.

Conticelli, S., Francalanci, L., and Santo, A. P. (1991). Petrology of final-stage Latera lavas (Vulsini Mts.)*: mineralogical, geochemical and Sr-isotopic data and their bearing on the genesis of some potassic magmas in central Italy*. *J. Volcanol. Geoth. Res.* 46, 187–212. doi: 10.1016/0377-0273(91)90083-c

Conticelli, S., Laurenzi, M. A., Giordano, G., Mattei, M., Avanzinelli, R., Melluso, L., et al. (2010). Leucite-bearing (kamafugitic/leucititic) and-free (lamproitic) ultrapotassic rocks and associated shoshonites from Italy: constraints on petrogenesis and geodynamics. *Journal of the Virtual Explorer* 36:20.

Conticelli, S., Manetti, P., Peccerillo, A., and Santo, A. (1986). Caratteri petrologici delle vulcaniti potassiche italiane: considerazioni genetiche e geodinamiche. *Mem. Soc. Geol. It.* 35, 775–783.

Di Paola-Naranjo, R., Baroni, M. V., Podio, N. S., Rubinstein, H. R., Fabani, M. P., Badini, R. G., et al. (2011). Fingerprints for main varieties of Argentinean wines: Terroir differentiation by inorganic, organic, and stable isotopic analyses coupled to chemometrics. *J. Agr. Food Chem.* 59, 7854–7865. doi: 10.1021/jf2007419

Durante, C., Baschieri, C., Bertacchini, L., Cocchi, M., Sighinolfi, S., Silvestri, M., et al. (2013). Geographical traceability based on 87Sr/86Sr indicator: A first approach for PDO Lambrusco wines from Modena. *Food Chem.* 141, 2779–2787. doi: 10.1016/j.foodchem.2013.05.108

Durante, C., Bertacchini, L., Bontempo, L., Camin, F., Manzini, D., Lambertini, P., et al. (2016). From soil to grape and wine: Variation of light and heavy elements isotope ratios. *Food Chem.* 210, 648–659. doi: 10.1016/j.foodchem.2016.04.108

Durante, C., Bertacchini, L., Cocchi, M., Manzini, D., Marchetti, A., Rossi, M. C., et al. (2018). Development of 87Sr/86Sr maps as targeted strategy to support wine quality. *Food Chem.* 255, 139–146. doi: 10.1016/j.foodchem.2018.02.084

Epova, E. N., Bérail, S., Séby, F., Vacchina, V., Bareille, G., Médina, B., et al. (2019). Strontium elemental and isotopic signatures of Bordeaux wines for authenticity and geographical origin assessment. *Food chem.* 294, 35–45. doi: 10.1016/j.foodchem.2019.04.068

Fortunato, G., Mumic, K., Wunderli, S., Pillonel, L., Bosset, J. O., and Gremaud, G. (2004). Application of strontium isotope abundance ratios measured by MC-ICP-MS for food authentication. *J. Anal. At. Spectrom.* 19, 227–234. doi: 10.1039/b307068a

Horn, P., Schaaf, P., Holbach, B., Hölzl, S., and Eschnauer, H. (1993). 87Sr/86Sr from rock and soil into vine and wine. *Zeitschrift für Lebensmittel-Untersuchung und Forschung* 196, 407–409. doi: 10.1007/bf01190802

IUSS (2006). *World Reference Base for Soil Resources 2006*. World Soil Resources Reports, 103.

Jackson, R. S. (2008). *Wine Science: Principles and Applications*. Netherland: Elsevier Inc.

Kawasaki, A., Oda, H., and Hirata, T. (2002). Determination of strontium isotope ratio of brown rice for estimating its provenance. *Soil Sci. Plant Nutr.* 48, 635–640. doi: 10.1080/00380768.2002.10409251

Kelly, S., Heaton, K., and Hoogewerff, J. (2005). Tracing the geographical origin of food: The application of multi-element and multi-isotope analysis. *Trends Food Sci. Tech.* 16, 555–567. doi: 10.1016/j.tifs.2005.08.008

Marchionni, S., Braschi, E., Tommasini, S., Bollati, A., Cifelli, F., Mulinacci, N., et al. (2013). High-precision 87Sr/86Sr analyses in wines and their use as a geological fingerprint for tracing geographic provenance. *J. Agr. Food Chem.* 61, 6822–6831. doi: 10.1021/jf4012592

Marchionni, S., Buccianti, A., Bollati, A., Braschi, E., Cifelli, F., Molin, P., et al. (2016). Conservation of 87Sr/86Sr isotopic ratios during the winemaking processes of 'Red' wines to validate their use as a geographic tracer. *Food Chem.* 190, 777–785. doi: 10.1016/j.foodchem.2015.06.026

Marra, F., Castellano, C., Cucci, L., Florindo, F., Gaeta, M., Jicha, B. R., et al. (2020a). Monti Sabatini and Colli Albani: the dormant twin volcanoes at the gates of Rome. *Sci. Rep. Vol.* 10:8666.

Marra, F., Jicha, B., Palladino, D. M., Gaeta, M., Costantini, L., and Di Buduo, G. M. (2020b). 40Ar/39Ar single crystal dates from pyroclastic deposits provide a detailed record of the 590–240 ka eruptive period at the Vulsini Volcanic District (central Italy). *J. Volcanol. Geotherm. Res.* 398:106904. doi: 10.1016/j.jvolgeores.2020.106904

Martin, G. J., Mazure, M., Jouitteau, C., Martin, Y. L., Aguile, L., and Allain, P. (1999). Characterization of the geographic origin of Bordeaux wines by a combined use of isotopic and trace element measurements. *Am. J. Enol. Viticul.* 50, 409–417.

Medini, S., Janin, M., Verdoux, P., and Techer, I. (2015). Methodological development for 87Sr/86Sr measurement in olive oil and preliminary discussion of its use for geographical traceability of PDO Nimes (France). *Food Chem.* 171, 78–83. doi: 10.1016/j.foodchem.2014.08.121

Metzeltin, S., and Vezzoli, L. (1983). Contributi alla geologia del Vulcano di Latera (Monti Vulsini, Toscana Meridionale- Lazio Settentrionale). *Mem. Soc. geol. It.* 25, 247–271.

Nappi, G., Capaccioni, B., Mattioli, M., Mancini, E., and Valentini, L. (1994). Plinian fall deposits from Vulsini volcanic district (central Italy). *Bull. Volcanol.* 56, 502–515. doi: 10.1007/s004450050059

Nappi, G., Renzulli, A., and Santi, P. (1991). Evidence of incremental growth in the Vulsinian calderas (central Italy). *J. Volcanol. Geotherm. Res.* 47, 13–31. doi: 10.1016/0377-0273(91)90098-k

Palladino, D. M., Simei, S., Sottili, G., and Trigila, R. (2010). Integrated approach for the reconstruction of stratigraphy and geology of Quaternary volcanic terrains: an application to the Vulsini Volcanoes (central Italy). *Geol. S. Am. S.* 464, 63–84.

Petrini, R., Sansone, L., Slejko, F. F., Buccianti, A., Marcuzzo, P., and Tomasi, D. (2015). The 87Sr/86Sr strontium isotopic systematics applied to Glera

vineyards: a tracer for the geographical origin of the Prosecco. *Food Chem.* 170, 138–144. doi: 10.1016/j.foodchem.2014.08.051

Roβmann, A., Haberhauer, G., Holzl, S., Horn, P., Pichlmayer, F., and Voerkelius, S. (2000). The potential of multielement stable isotope analysis for regional assignment of butter. *Eur. Food Res. Technol.* 211, 32–40. doi: 10.1007/s002170050585

Song, B. Y., Ryu, J. S., Shin, H. S., and Lee, K. S. (2014). Determination of the source of bioavailable Sr using 87Sr/86Sr tracers: a case study of hot pepper and rice. *J. Agr. Food Chem.* 62, 9232–9238. doi: 10.1021/jf503498r

Tescione, I., Marchionni, S., Casalini, M., Vignozzi, N., Mattei, M., and Conticelli, S. (2018). 87Sr/86Sr isotopes in grapes of different cultivars: A geochemical tool for geographic traceability of agriculture products. *Food Chem.* 258, 374–380. doi: 10.1016/j.foodchem.2018.03.083

Tescione, I., Marchionni, S., Mattei, M., Tassi, F., Romano, C., and Conticelli, S. (2015). A Comparative 87Sr/86Sr Study in Red and White Wines to Validate its Use as Geochemical Tracer for the Geographical Origin of Wine. *Proced. Earth Plan. Sci.* 13, 169–172. doi: 10.1016/j.proeps.2015.07.039

Thirlwall, M. F. (1991). Long-term reproducibility of multicollector Sr and Nd isotope ratio analysis. *Chem. Geol.* 94, 85–104. doi: 10.1016/s0009-2541(10)80021-x

Tommasini, S., Marchionni, S., Tescione, I., Casalini, M., Braschi, E., Avanzinelli, R., et al. (2018). *Strontium isotopes in biological material: A key tool for the geographic traceability of foods and humans beings in Behaviour of Strontium in Plants and the Environment*. Berlin: Springer, 145–166.

Turbeville, B. N. (1992). 40Ar/39Ar ages and stratigraphy of the Latera caldera, Italy. *Bull. Volcanol.* 55, 110–118. doi: 10.1007/bf00301124

Turbeville, B. N. (1993). Petrology and petrogenesis of the Latera caldera, central Italy. *J. Petr.* 34, 77–124. doi: 10.1093/petrology/34.1.77

Varekamp, J. C. (1979). Geology and petrology of the Vulsinian volcanic area (Latium, Italy). *Geol. Ultraiectina* 22, 1–384.

Vezzoli, L., Conticelli, S., Innocenti, F., Landi, P., Manetti, P., Palladino, D. M., et al. (1987). Stratigraphy of the Latera Volcanic Complex: proposals for a new nomenclature. *Period. Mineral.* 56, 89–110.

Vinciguerra, V., Stevenson, R., Pedneault, K., Poirier, A., and Hélie, J.-F. (2016). Strontium isotope characterization of wines from Quebec, Canada. *Food Chem.* 210, 121–128. doi: 10.1016/j.foodchem.2016.04.017

Voerkelius, S., Lorenz, G. D., Rummel, S., Quétel, C., Heiss, G., Baxter, M., et al. (2010). Strontium isotopic signatures of natural mineral waters, the reference to a simple geological map and its potential for authentication of food. *Food Chem.* 118, 933–940. doi: 10.1016/j.foodchem.2009.04.125

Vorster, C., Greeff, L., and Coetzee, P. P. (2010). The determination of 11B/10B and 87Sr/86Sr isotope ratios by quadrupole-based ICP-MS for the fingerprinting of South African wine. *S. Afr. J. Chem.* 63, 207–214.

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Carbon Isotope Discrimination ($\delta^{13}C$) of Grape Musts Is a Reliable Tool for Zoning and the Physiological Ground-Truthing of Sensor Maps in Precision Viticulture

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Carbon stable isotope composition of berry must at harvest ($\delta^{13}C$) is an integrated assessment of plant water status during grape (*Vitis vinifera* L.) berry ripening. Measurement of $\delta^{13}C$ of grape juice is proposed as an alternative to traditional measurements of water status to capture the spatial variability of physiological response at the vineyard scale, i.e., zoning. We performed samplings at four different locations in California, United States, with three different cultivars of table and wine grapes (Cabernet Sauvignon, Merlot, Crimson-Seedless). Leaf physiology (photosynthesis, A_N, stomatal conductance, g_s) and stem water potentials (Ψ_{stem}) were routinely measured. The $\delta^{13}C$ was measured at harvest and strong relationships were found between Ψ_{stem} (R^2 = 0.71), stomatal conductance (R^2 = 0.71), net carbon assimilation (R^2 = 0.59) and WUEi (R^2 = 0.53). The role of leaf nitrogen on the signal was assessed by evaluating relationships between leaf nitrogen and WUE_i (R^2 = 0.54), Ci/Ca (R^2 = 0.51), $\delta^{13}C$ (R^2 = 0.44), and Ψ_{stem} (R^2 = 0.37). Although nitrogen can be among the environmental factors able to affect the $\delta^{13}C$ signal, this difference is only observable when variability in *N* is very large, by pooling different vineyards/varieties, but not at the within-vineyard scale. The utility of $\delta^{13}C$ was further tested and measured on grape berries sampled on an equidistant grid in a 3.5 ha vineyard where Ψ_{stem} was also measured throughout the field season and used to delineate management zones. Physiological measurements and grape composition were correlated to soil electrical resistivity and satellite-derived vegetation index. The two management zones obtained by $\delta^{13}C$ or Ψ_{stem} were spatially similar at 67% and allowed to separate the harvest in two pools having statistically different grape composition (soluble solids, organic acids, and anthocyanin profiles). Zoning by $\delta^{13}C$ performed as well as zoning by Ψ_{stem} to separate grape phenolic composition, e.g., for selective harvest. Our results provided evidence that $\delta^{13}C$ of grape must is a reliable and repeatable assessor of plant water status and gas exchange in vineyard systems that are crucial for zoning vineyards, even when irrigated, and for ground-truthing sensor maps in precision viticulture.

Keywords: water, precision agriculture, anthocyanins, nitrogen, terroir, irrigation, zoning, selective harvest

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INTRODUCTION

The majority of the world's viticulture areas are in arid and semi-arid regions where vineyards receive annual precipitation amounts below 700 L m^{-2} (Flexas et al., 2010). The growth and development of the grapevine usually correspond to the dry spring and summer months in these regions where there is little to no precipitation. The water consumption in vineyards ranges between 300 mm to 700 mm and this is greater than annual precipitation received in arid and semi-arid regions of the world (Medrano et al., 2015). Therefore, in these regions, commercial grapevine production relies on supplemental irrigation. The sustainability of grapevine production is there under threat due to growing water scarcity, rising global temperatures, and suboptimal irrigation strategies (White et al., 2006; Diffenbaugh and Scherer, 2013; Bustan et al., 2016; Bonfante et al., 2018). This problem is especially meaningful considering that grapevine is economically viable in soils with reduced fertility not well suited to other crops and that Mediterranean ecosystems adapted to grapevine are global biodiversity hotspots (Myers et al., 2000).

Agriculture is the largest user of fresh water, accounting for 99% of the global consumptive water footprint (Hoekstra and Mekonnen, 2012). This is confirmed in California, where agriculture irrigation consists of 74% of total freshwater use (Maupin et al., 2014). Under standard cultural practices in California vineyards, 140 to 220 L of irrigation water is used to produce 1 kg of wine grape (Martínez-Lüscher et al., 2017). Optimization of irrigation is not only important for environmental sustainability but also because it directly affects the yield and composition of grapes and wines (Castellarin et al., 2007; Brillante et al., 2017, 2018a). The grapevine agronomic performance under mild water deficits is well documented (Chaves et al., 2010). However, variability in the physical environment at the growing site affects grapevine water status in space and time within the same vineyard (Brillante et al., 2016a) resulting in locally inadequate irrigation within uniformly irrigated blocks (Brillante et al., 2016a, 2017) and variability problems can be exacerbated by inefficiencies in the irrigation system. This usually results in spatial differences in yield and/or grape composition at harvest (Acevedo-Opazo et al., 2008; Taylor et al., 2010; Brillante et al., 2017).

Water savings and better agronomic performances could be obtained by reducing spatial heterogeneity in plant water status (Sanchez et al., 2017), avoiding local over-irrigation, and by better tailoring irrigation strategies (Brillante et al., 2018a). Routine measurements of plant water status in the field are time-consuming and they must be constrained to few locations and time points, thus they do not easily allow a spatialized approach. Current remote sensing (Gutiérrez et al., 2018; Kustas et al., 2019) and modeling methodologies (Brillante et al., 2016b) for zoning and mapping plant water status in precision agriculture need a validation measurement that is reliable and easy to measure in multiple locations. Carbon stable isotope composition in grape musts can address this issue, as it is rapid to measure and has been proposed as a reliable continuous integrator of plant water status throughout the ripening period (Gaudillère et al., 2002). In short, the heavier stable carbon isotopes are discriminated through the difference in binary and ternary diffusivity and ribulose 1,5-diphosphate carboxylase oxygenase kinetic constants of $^{13}CO2$ and $^{12}CO2$. When stomata are closed because of water deficit this kinetic preference is reduced, as the $^{13}CO2/^{12}CO2$ ratio increases in the sub-stomatal cavity. The resultant photoassimilates are then enriched in ^{13}C. Water deficits are the determining factor affecting stomatal conductance and by extension the proportion of ^{13}C assimilated (Farquhar et al., 1989). Measurements of carbon isotopic discrimination, $\delta^{13}C$, can be performed on different organs and growing stages for different purposes. The use of this analysis on grape musts offers operational advantages for estimating overall water status during the ripening period (Bchir et al., 2016), as it can be performed on the same substratum used for monitoring ripening, although not offering an instantaneous evaluation useful to schedule water as for the Scholander pressure chamber. Analyses of berry must $\delta^{13}C$ can be performed once at the end of the season to characterize the spatial pattern of the water status at the field scale (Herrero-Langreo et al., 2013), or to better understand the response of the grapevine in a given vintage (Brillante et al., 2018b), or for comparing the water use efficiency of accessions in breeding programs. Although the direct relationship between $\delta^{13}C$ and classic reporting of plant water status by leaf or stem water potential (Ψ) was shown by previous studies across the world (see Brillante et al., 2018b, for a recent meta-analysis), the relationship with gas exchange was much less investigated and when considering the measurement of $\delta^{13}C$ on leaves contrasting results were also reported (Poni et al., 2009; Bchir et al., 2016). The relationships between grapevine predawn water potential, Ψ_{pd} and $\delta^{13}C$ were shown to vary in the intercept across cultivars and locations, but not in the slope (Brillante et al., 2018b), offering a way to translate isotope composition values to grapevine Ψ in relative, but also rising the need for calibration in new conditions, as in California. Additional work for understanding the relationship between plant Ψ and $\delta^{13}C$ of grape musts is needed, and also extending the study to evaluate the link between $\delta^{13}C$ of musts and leaf gas exchange parameters that are not frequently found in the literature.

Considering the need for a measurement that can assess water status of grapevine reliably, rapidly and cost-effectively, the overarching aim of this work was to conduct a multi-area calibration of $\delta\ ^{13}C$ to grapevine Ψ and leaf gas exchange parameters to better understand the $\delta\ ^{13}C$ signal in viticulture, across economically important cultivars used in wine and table grape production. The specific objective of this study was to apply this knowledge to assess if intra-vineyard variability in water status and gas-exchange could be assessed by $\delta^{13}C$, and use $\delta^{13}C$ to delineate management zones for selective harvest or site-specific management, or as a method to provide an effective and reliable physiological ground-truthing for sensor maps (e.g., remotely sensed vegetation indexes, soil electrical resistivity). This is crucial for vine physiology-based zoning, and therefore, to implement site-specific strategies in precision viticulture (e.g., selective harvest, design of variable-rate irrigation systems etc.).

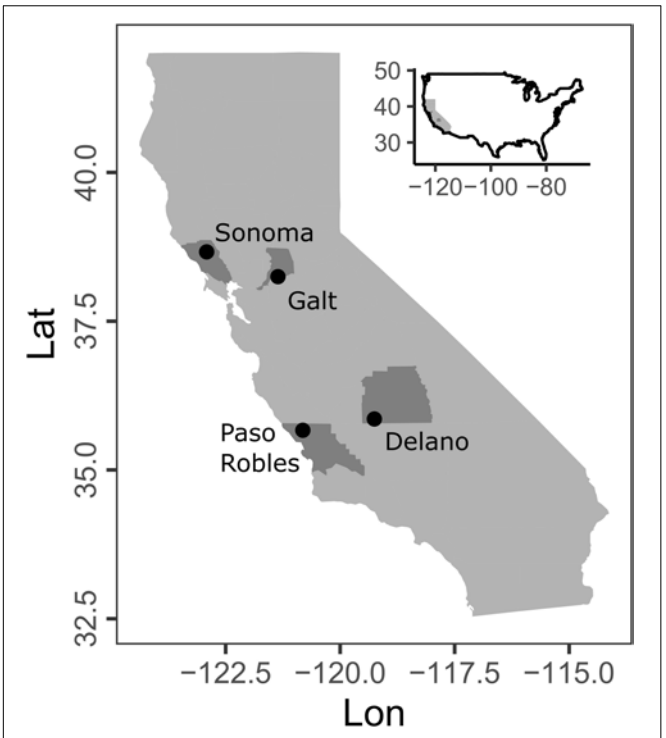

FIGURE 1 | Geographical locations of the experimental vineyards in the study.

MATERIALS AND METHODS

Experimental Sites and Plant Material

The experiment was conducted during 2016 at four different experimental sites located across the state of California (**Figure 1**). The characteristics of these fields are reported in **Table 1**. In Sonoma and Galt, vineyards were planted on Cabernet-Sauvignon grafted on 110R (*V. berlandieri* Planch. × *V. rupestris* Scheele) and 1103P (*V. berlandieri* Planch. × *V. rupestris* Scheele) respectively, trained on a high-quadrilateral trellis, and spur pruned on two bilateral cordons. In Paso Robles, the vineyard was planted on Merlot Noir, grafted on 1103P, and trained on a Vertically-Shoot-Positioned (VSP) trellis and spur pruned on a unilateral cordon (see Martínez-Lüscher et al., 2019 experiment 3 for a more detailed description of this vineyard). In Delano, the vineyard was planted on Crimson-Seedless grafted on Freedom (1613-59 × Dog Ridge 5), cane pruned and on gable trellis.

Physiological measurements and berry samplings for $\delta^{13}C$ and skin anthocyanin analysis were performed on experimental units composed of ten grapevines each and spatially distributed across each vineyard block according to a stratified random sampling based on multivariate clustering of electrical resistivity, and Normalized Difference Vegetation Index (NDVI) measured as described in the next section. In Sonoma, experimental units were instead located on a 33 m equidistant grids and composed by 5 vines each (see Brillante et al., 2017 for a more detailed description of this vineyard).

Chemical Analysis

Carbon Isotope Composition of Musts

Carbon stable isotope composition was measured in musts of mature grapes obtained from one composite sample of 100 berries, following the protocol described by Gaudillère et al. (2002). Berries were randomly sampled from multiple clusters and collected without pedicel. They were stored in ice and later crushed in the laboratory to obtain the juice. The juice was spun twice, approximately 40 ml were spun at 2000 × g and 1 ml aliquot was collected in a smaller tube and centrifuged again at 14,119 × *g* to further remove suspended solids. Then 5ul of the clear liquid was inserted in thin capsules, dried overnight at 60°C, and encapsulated using tweezers. Isotopic analyses were performed at the UC Davis Stable Isotope facility, using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, United Kingdom). Samples were combusted at 1080°C in a reactor packed with chromium oxide and silvered copper oxide. Following combustion, oxides were removed in a reduction reactor (reduced copper at 650°C). The helium carrier then flew through a water trap (magnesium perchlorateand phosphorous pentoxide). CO_2 was retained on an adsorption trap until the N_2 peak was analyzed; the adsorption trap was then heated releasing the CO_2 to the IRMS. Samples were interspersed with several replicates, in addition to at least two

TABLE 1 | General characteristics of the experimental design and vineyards.

Vineyard	Cultivar	Rootstock	Trellis system	Pruning	Experimental design	Experimental Units (#)	Cultural practices
Sonoma	Cabernet-Sauvignon	110R	High-quadrilateral	Spur	Equidistant grid	35	None
Galt	Cabernet-Sauvignon	1103 P	High-quadrilateral	Spur	Stratified random sampling	16	Variable-rate irrigation; leaf removal; zone control
Paso Robles	Merlot Noir	1103 P	Vertical Shoot Positioning	Spur	Stratified random sampling	24	Leaf removal; Shoot thinning; Leaf removal * Shoot; thinning; zone control
Delano	Crimson Seedless	Freedom	Gable	Cane	Stratified random sampling	16	Deficit irrigation 0.65 ETc, 0.8 ETc; zone control (1 ETc)

different laboratory standards, and the standard deviation was lower than 0.2 per thousand, and conform to the long-term standard deviation of the lab. All results are expressed in delta notation, as calculated in equation (1)

$$\delta^{13}C = \left[\frac{R_{sample}}{R_{std}} - 1\right] \times 1000 \quad (1)$$

where R_{sample} and R_{std} are the absolute $^{13}C/^{12}C$ ratios for sample and standard. The values of $\delta^{13}C$ are reported in parts per thousand respect to the Vienna Pee Dee Belemnite (VPDB) international reference.

Grape Berry Anthocyanin Extraction and Analysis

Grape berry skin anthocyanins were analyzed on 20 berries randomly sampled from each per experimental unit at maturity. Berry skins were gently peeled using a scalpel and then freeze-dried (model 7810014/7385020, Labconco, Kansas City, MO, United States). Dry skin weights were recorded after lyophilization, and then the skin tissues were powdered with a tissue lyser (MM400, Retsch, Mammelzen, Germany). Ground dry skin (50 mg) was weighed and extracted overnight at 4°C with 1 mL of methanol:water:7 M hydrochloric acid (70:29:1). Extracts were filtered with PTFE membrane filters (0.45 μm, VWR, Seattle, WA, United States), and transferred into HPLC vials before injection. The HPLC-DAD analyses of anthocyanins were performed with an Agilent 1260 (Santa Clara, CA, United States) with a LiChrospher 100, 250 mm × 4 mm with a 5 μm particle size and a 4mm guard column of the same material. HPLC gradient was the same as Ritchey and Andrew (1999).

Leaf Nitrogen Content and Plant Biomass

Vine leaf blades were collected to determine nutrient status at anthesis in each of the experimental vineyards. Fifty leaves in a position opposite a cluster were collected per experimental unit. Leaf-blades were rinsed in distilled water, dried at 65°C for 48 h and ground to pass through a 0.425 m sieve. Total nitrogen was determined by Dellavalle, Inc., Fresno, CA, United States via automated combustion analysis, method B-2.20, (Gavlak et al., 1994).

Plant biomass was estimated by collecting and weighing the wood after pruning four grapevines per experimental unit.

Supportive Analysis

Spatial Data Acquisition and Terrain Analysis

A digital elevation model was acquired using a differentially correct GPS (post-processing accuracy 2–5 cm in all directions) TRIMBLE Pro 6T DGNSS receiver (Trimble Inc., CA, United States). A terrain analysis was then performed using SAGA GIS v.2.1.2. (Conrad et al., 2015) to compute aspect, slope, and wetness index, SAGA WI (Conrad et al., 2015).

Soil Electrical Resistivity

At the beginning of the growing season, soil electrical resistivity measurements were performed using an EM38-MK2 (Geonics Limited) soil electrical conductivity meter. Measurements were performed in vertical dipole orientation at 0.75 m (Shallow ER) and 1.50 m (Deep ER). The sensor of the instrument was calibrated according to the manufacturer's instructions to minimize the errors before the survey. The instrument was placed on a non-conductive PVC sled at an approximately 15 cm height above the ground and pulled by an all-terrain vehicle along the inter-rows at a distance of ∼2.5 m to avoid interference phenomena with the vehicle. The use of the PVC sled made it possible to keep the instrument at a constant distance from the soil surface, making data acquisition easier.

Normalized Difference Vegetation Index

At the beginning of flowering (modified E-L scale #19), canopy reflectance as NDVI was measured using a Crop Circle AS430 (Holland Scientific Inc.). The data stream was logged at 1 Hz to a GeoScout datalogger (Holland Scientific Inc.) and geolocated with a WAAS-enabled Garmin 18× GPS (Garmin Ltd.).

Solar-Noon Stem Water Potential

Plant water status was measured as stem water potentials (Ψ_{stem}). For each experimental unit and sampling instance, three (in Sonoma) to six leaves (anywhere else) from the middle section of main shoot axis were covered with a reflecting zip-top mylar bag for 2h. Around solar noon (12:30-15:30h), leaves were cut with a razor blade and immediately measured in a pressure chamber (Model 615, PMS instruments Co., OR, United States).

Leaf Gas Exchange

Leaf gas exchange was measured at solar noon (12:30-15:30h) using a portable infrared gas analyzer CIRAS-3 (PP Systems, Amesbury, MA, United States), featuring a broad-leaf chamber with 4.5 cm^2 window size. In each experimental unit, three sun-exposed leaves from three grapevines and on an intermediate position of a main shoot were measured; plants and leaves randomly varied between dates. To mitigate the effect of time, the order in which experimental units were measured was randomly assigned from one measurement date to another. Assimilation rate (A_N, μmol CO^2 m^{-2} s^{-1}) and stomatal conductance (g_s, mmol H_2O m^{-2} s^{-1}) were obtained by measurement of inlet and outlet CO_2 and H_2O relative concentration. Intrinsic water use efficiency (WUEi) was calculated as the ratio between A_N and g_s (and then expressed in μmol CO_2 $mmol^{-1}$ H_2O). The cuvette was oriented perpendicularly to sunlight, which was always in saturating conditions (average of internal PAR > 1900 μmol m^{-2} s^{-1}). Measurements were taken at 60% relative humidity, with a CO_2 concentration of 390 μmol CO_2 mol^{-1}, and using a flow to the chamber of 300 mL min^{-1}.

Statistical Analysis

Statistical analysis was performed in R 3.5.1 (R Core Team, 2018). Generally in the text, the term significant is used to indicate p-value < 0.05.

When used, integrals of Ψ_{stem} and leaf gas exchange were calculated using the composite trapezoid rule, then divided by the time range to have values more easily comparable to individual date measurements. Correlations between parameters in the same vineyard were assessed according to a modified t-test for spatial processes (Dutilleul, 1993).

Maps of the $\delta^{13}C$ and Ψ_{stem} were obtained by linear interpolation with *x*, *y*, *z* coordinates, and prediction error on unseen locations was estimated through bootstrap. Zoning was performed by k-means, and the similarity between $\delta^{13}C$ and Ψ_{stem} zones evaluated by the Rand index.

Anthocyanin maps were performed using universal block kriging with variables of interest being linearly dependent on *x*, *y*, *z* coordinates, and block size 33 m * 33 m. Variogram shape was assessed by multiple tests and comparisons using cross-validation. The gstat package (v. 1.1-6) was used for this purpose (Pebesma, 2004). Comparison of means across management zones was carried-out using generalized least-square ANOVA to account for spatial dependency.

The stability of the linear regression estimates (slope and R^2) between $\delta^{13}C$ and Ψ_{stem} with an increasing number of samples was assessed using a resampling routine with replacement of the whole season data set. The exercise aim was to simulate what could have happened to the $\delta^{13}C \sim \Psi_{stem}$ relations if one or more sampling dates would have been missing from the dataset. For this purpose linear regression explaining Ψ_{stem} integrals, mean or minimum as a function of $\delta^{13}C$ was fitted to the subset with a variable number of observations from a minimum of 3 to a maximum of total observations – 1 (6). Each resample subset was composed of unique observations and the original temporal order was conserved.

RESULTS

Relationships Between Ψ_{stem} and Leaf Gas Exchange

A large variation was observed in the Ψ_{stem} when all cultivars (Cabernet Sauvignon, Merlot, Crimson Seedless) were pooled, in which the values ranged from −0.66 to −1.8 MPa covering a comprehensive range of plant water status for grapevine. Likewise, we also observed considerable variation in leaf gas exchange (wine grapes only). Stomatal conductance (g_s) ranged from 7 to 311 mmol H_2O m^2 s^{-1}, and net carbon assimilation (A_N) ranged from 1.5 to 21 μmol CO_2 m^2 s^{-1}. Intrinsic water use efficiency (WUEi) ranged from 0.04 to 0.29 (μmol CO_2 $mmol^{-1}$ H_2O).

Stem water potential and leaf gas exchange parameters were linearly related across the three wine grape locations and two cultivars over which they were measured. **Figure 2** presents single date averages across all experimental units. Within our range of data, the general response to an increase in Ψ_{stem} was a significant linear increase in both g_s and A_N. Conversely, this general response was reversed with WUEi in which as Ψ_{stem} increased, the WUEi significantly and linearly decreased. Some difference is observable between Cabernet and Merlot in the relationships of Ψ_{steam} with g_s and A_N, but not with WUE_i with Merlot having general lower g_s and A_N at equivalent Ψ_{stem}. We report these results in **Supplementary Figure 1**, but it is important to note that the environment was not controlled and the varieties were located in different growing regions and differentially managed,

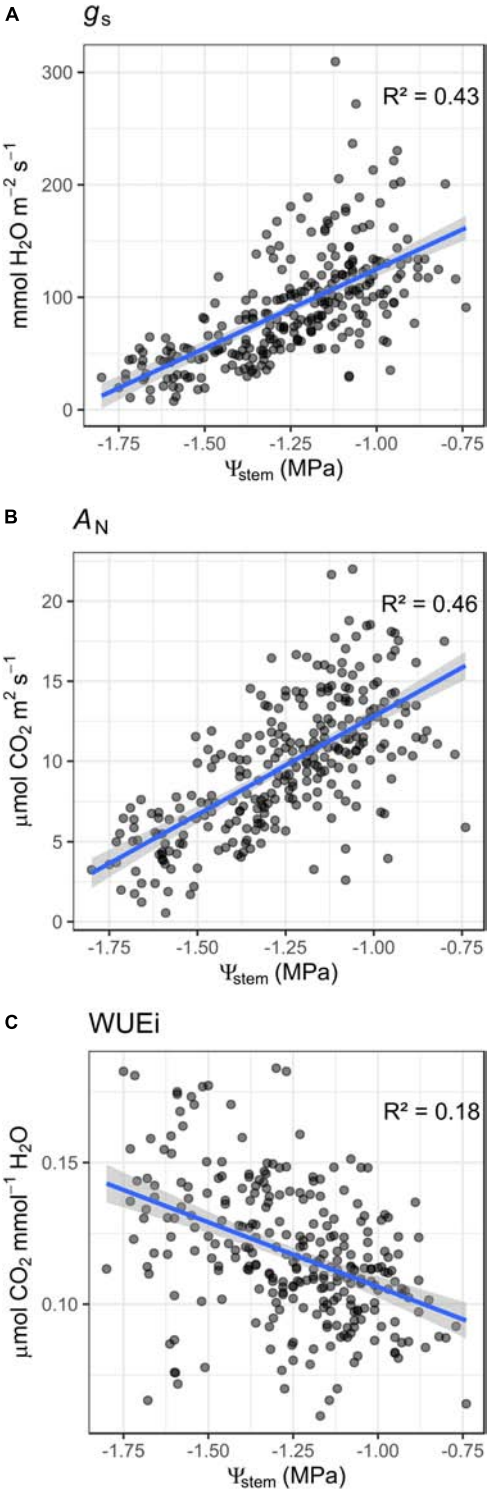

FIGURE 2 | Relationship between stem water potential and leaf gas exchange in grapevine across three different vineyards in three different viticulture areas and two different varieties (Merlot and Cabernet-Sauvignon). Each point is an aggregate of minimum 3 measurements on different leaves and plants within a single experimental unit. **(A)** relationship with stomatal conductance; (g_s) **(B)** relationship with A_N **(C)** relationship with intrinsic water use efficiency (WUEi). Blue line is a linear regression line; in gray is the confidence interval.

TABLE 2 | Summary statistics of the $\delta^{13}C$ data measured on grape juice samples

		Min. $\delta^{13}C$ (‰)	Median $\delta^{13}C$ (‰)	Mean $\delta^{13}C$ (‰)	Max $\delta^{13}C$ (‰)	Observations #
	Delano	−26.9	−26	−26.1	−24.8	16
	Galt	−25.3	−23.9	−23.6	−23.1	16
Vineyard	Paso	−26.7	−24.8	−24.7	−23.8	24
	Sonoma	−26.8	−25	−25.1	−23.7	35
	Cabernet	−26.8	−24.6	−24.6	−23.1	51
Variety	Crimson	−26.9	−26	−26.1	−24.8	16
	Merlot	−26.7	−24.8	−24.7	−23.8	24
All fields		−26.9	−24.9	−24.9	−23.1	91

therefore limiting our ability to interpret this difference between cultivars from a physiological standpoint.

Relationships Between Plant Water Status, Leaf Gas Exchange and $\delta^{13}C$ of Grape Musts

We observed a wide range in $\delta^{13}C$ values (−23.08 to −26.79 ‰), reflecting the variability we measured in plant water status and leaf gas exchange. The results are reported in **Table 2**. Compared to other $\delta^{13}C$ values reported in the literature, values are included in the mid of the range as summarized in Brillante et al. (2018b). They show values higher (indicating less stress) than the ones reported in Gaudillère et al. (2002), for Bordeaux Cabernets and Merlot; similar to the ones reported in Guix-Hébrard et al. (2007), for Shiraz in Languedoc, FR; and lower (indicating more stress) than the ones reported in Brillante et al. (2018b) for Chardonnay in Burgundy, FR.

There was a strong inverse linear correlation between mean Ψ_{stem} measured across the growing season and $\delta^{13}C$ measured on grapes at harvest, **Figure 3**. The relationship between leaf gas exchange (not available for Crimson Seedless) and $\delta^{13}C$ was further confirmed by regression analysis. There was a positive relationship between season integrals of g_s or A_N with $\delta^{13}C$ (**Figures 4A,B**). Likewise, the relationship with integrals of WUEi and $\delta^{13}C$ was also evident, albeit in the inverse direction (**Figure 4C**). **Supplementary Figure 2** shows differences between the two varieties in the relationships between the means of the leaf gas exchange variables and $\delta^{13}C$, although the intercept estimates are different the slope estimates are similar. The same limits observed in the interpretation of **Supplementary Figure 1** apply here.

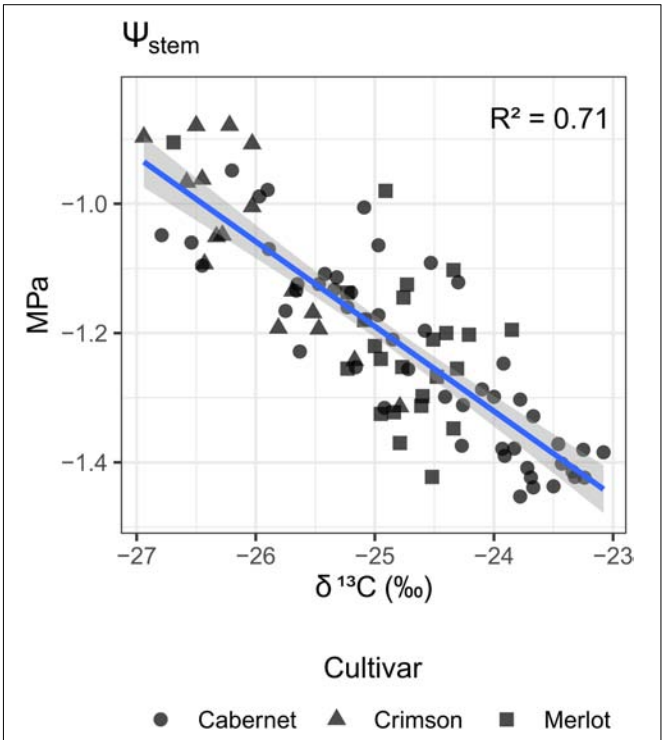

FIGURE 3 | Relationship between $\delta^{13}C$ of grape musts at harvest and seasonal means of Ψ_{stem} across four different vineyards in four different viticulture areas and three different varieties (Merlot, Cabernet-Sauvignon, Crimson-Seedless). Shape of point is mapped to the cultivar. Blue line is a linear regression line; in gray is the confidence interval.

Leaf Nitrogen Concentration and Its Relationship to Gas Exchange and δ13C

Leaf nitrogen concentration was significantly and positively related to WUEi (**Figure 5A**). Conversely, leaf nitrogen concentration was significantly and negatively related to Ci/Ca. (**Figure 5B**). We also observed a significant positive relationship between leaf nitrogen concentration and $\delta^{13}C$ (**Figure 5C**) when Cabernet-Sauvignon and Crimson Seedless data were pooled (nitrogen data were not available for the Merlot vineyard). The leaf nitrogen concentration was directly related to Ψ_{stem} whereas the total nitrogen concentration increased, Ψ_{stem} decreased (**Figure 5C**).

Comparing $\delta^{13}C$ and Plant Water Status to Delineate Management Zones for Selective Harvest in Precision Viticulture

Relationships Between Sensed Site Characteristics and Whole Grapevine Physiology

The physical variability of a producing vineyard was assessed by proximal sensing soil electrical resistivity, canopy reflectance, and by interpolating elevation, slope, and aspect from GPS

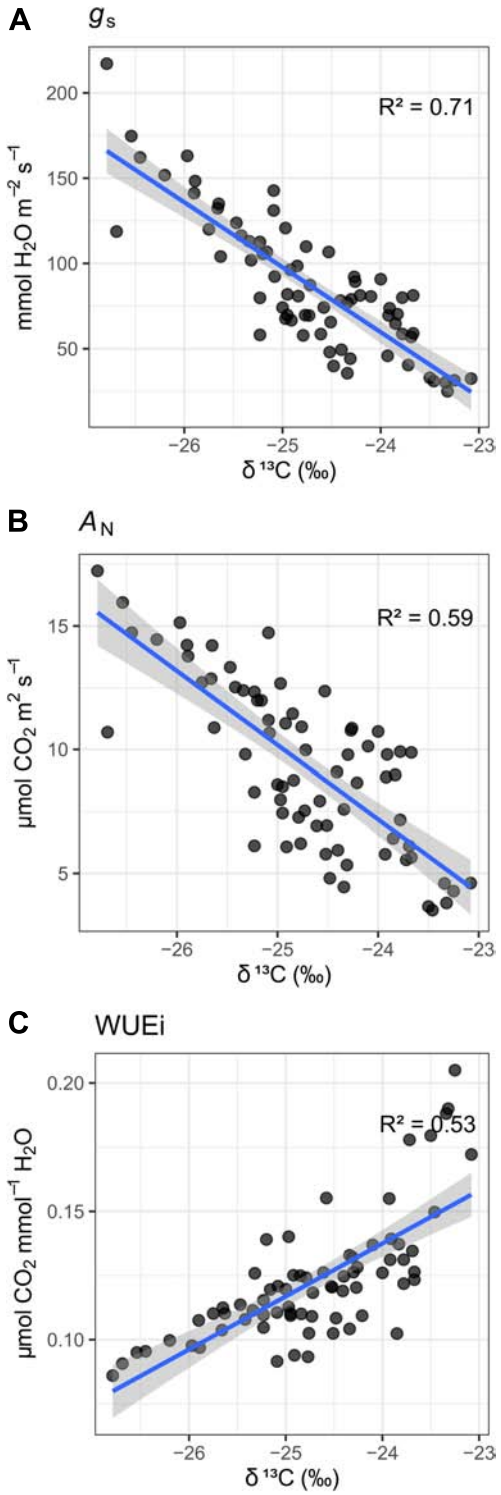

FIGURE 4 | Relationship between $\delta^{13}C$ of grape musts at harvest and seasonal integrals of leaf gas exchange across three different vineyards in three different viticulture areas and two different varieties (Merlot and Cabernet-Sauvignon). **(A)** relationship with stomatal conductance (g_s), **(B)** 4 relationship with A_N; **(C)** relationship with intrinsic water use efficiency (WUEi). Blue line is a linear regression line; in gray is the confidence interval. All p-values < 1e–12.

data obtained in-situ. The shallow (0–0.75 m) and deep (0–1.50 m) soil electrical resistivity (ER), and the canopy reflectance are presented in **Figure 6**. In each map, experimental units where plant measurements are represented by a black circle. At this research site, soil units varied with elevation indicating a toposequence. There was a significant relationship between shallow and deep soil ER to absolute elevation measured ($r = 0.73$, and $r = 0.70$, respectively). Furthermore, soil ER was well correlated to plant water status and leaf gas exchange. It is worth noting that shallow ER (0–0.75 m) was more informative of plant conditions than deep ER (0–1.5 m). Specifically, shallow ER was significantly correlated to Ψ_{stem}, $\delta^{13}C$, g_s, A_N, and WUEi, and deep ER was significantly correlated to Ψ_{stem} only. The SAGA WI was significantly correlated to Ψ_{stem}, but not to $\delta^{13}C$. The NDVI was positively correlated to the topographic wetness index but not to the SAGA WI or season-long Ψ_{stem} but was positively correlated to pruned wood weight. However, wood weight was significantly and inversely correlated to $\delta^{13}C$, g_s, and WUEi. Correlations between plant measurements and sensor data are reported in **Table 3**.

Interpolating Plant Water Status Deriving Management Zones

We interpolated plant water status by the use of Ψ_{stem} or $\delta^{13}C$. **Figure 7A** presents the map of plant water status by interpolating $\delta^{13}C$ measured from the berries sampled in the experimental units (black dots in the figure). **Figure 7B** presents the map of plant water status obtained with interpolation of season-long Ψ_{stem} integrals (7 measurement dates). For $\delta^{13}C$ the Root Mean Square Error, RMSE, in the bootstrap validation analysis was 0.63 ‰ with $R^2 =$ 0.54, and for Ψ_{stem} for, the RMSE was 0.1 MPa with an $R^2 = 0.56$. The projections of the **Figures 7A,B** look similar in their main trend with the North-West side of the vineyard displaying more water stress than the South-East side in either Figure. This result confirms the fundamental relation between $\delta^{13}C$ and Ψ_{stem} shown in **Figure 3**, which was also established with data from this vineyard. Zoning of plant water status in two management zones for selective harvest purposes was obtained by k-means clustering applied to the underlying data of **Figures 7A,B**. Zones were denominated "Severe" and "Moderate" water stress, and presented in **Figure 7C**, $\delta^{13}C$ derived, and 7d, Ψ_{stem}). The similarity of management zones whether calculated from **Figure 7A** to derive **7C** or from **Figure 7B** to derive **7D** was 0.67 as measured by the Rand index (Rand index values range between 0 and 1, for none too exact similarity).

Selective Harvest Zoning Using $\delta^{13}C$

Grape anthocyanin composition and profile varied greatly across the field as shown in **Figure 8**, and patterns closely reflected variability in water status. Assessment of variability in grape composition for selective harvest was conducted dividing the samples according to the location of experimental units within the zones in **Figures 7C,D**. This resulted in a total of four classes: 2 zones as separated by $\delta^{13}C$ and 2 management zones as separated by Ψ_{stem}. The differences

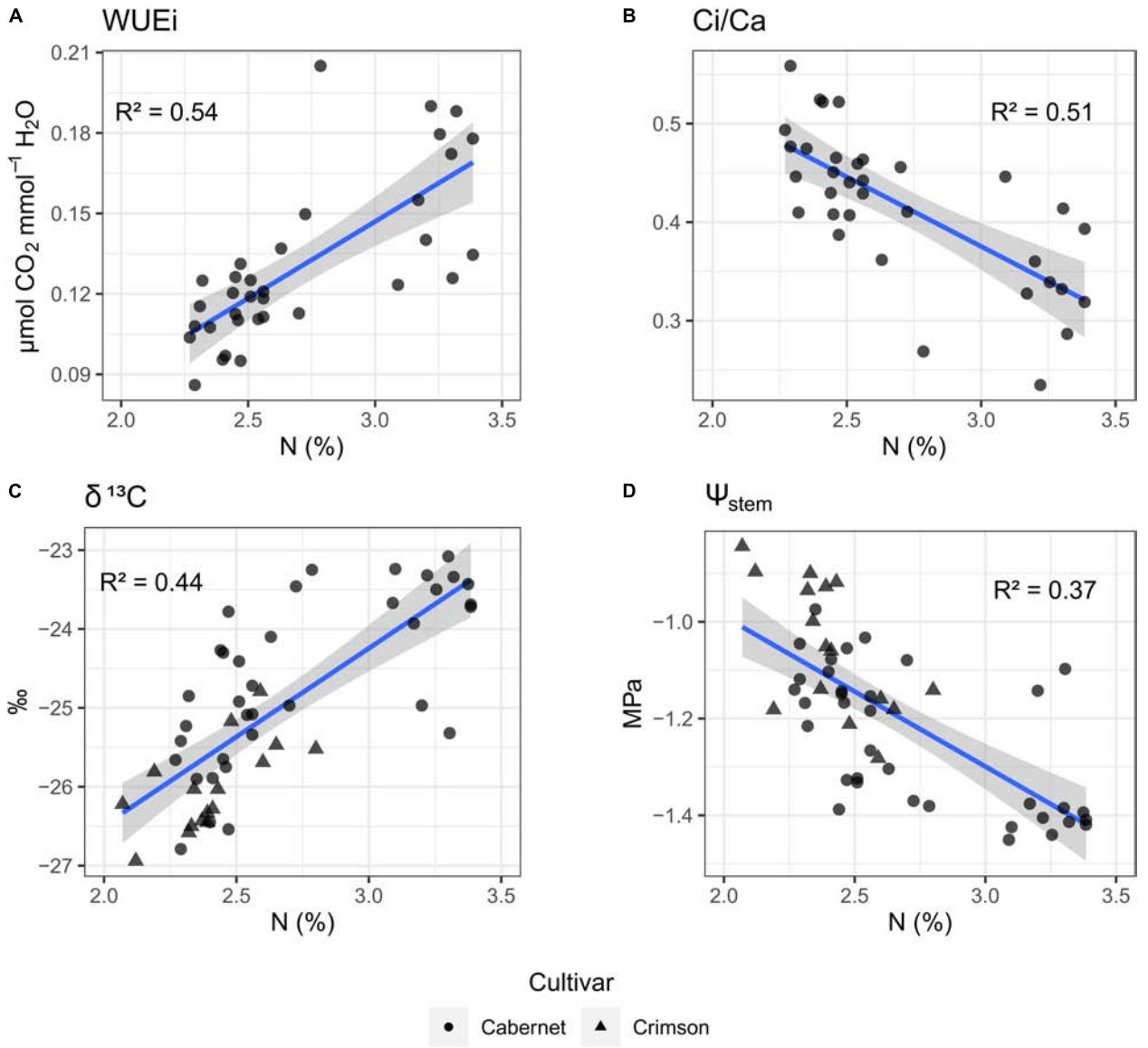

FIGURE 5 | Relationship between Nitrogen content measured in leaf blades, and **(A)** seasonal integrals of WUEi, **(B)** seasonal integrals of Ci/Ca, **(C)** $\delta^{13}C$ of musts, and **(D)** seasonal integrals of Ψ_{stem}. Shape of points is mapped to cultivar. Blue line is a linear regression line; in gray is the confidence interval.

in anthocyanin amount and composition of grape berry as separated by these classes were analyzed with analysis of variance to compare the efficiency of $\delta^{13}C$ or Ψ_{stem} for selective harvest. The results are presented in **Figure 9**. The total amount of anthocyanins, the amount of di-hydroxylated and tri-hydroxylated forms per berry basis, and the ratio between these forms was compared. The severe water stress zone had a significantly lower amount of anthocyanins on a per berry basis, a lower amount of tri-hydroxylated and of di-hydroxylated anthocyanins as well as a higher ratio between of the two hydroxylase forms compared to the moderate water stress zone. The use of $\delta^{13}C$ or Ψ_{stem} performed, similarly, in segregating harvest zones. The preference of $\delta^{13}C$ or Ψ_{stem} did not result in a significant difference in the anthocyanin amount or composition if one or the other plant water status assessment method was utilized.

Effect of Sampling Granularity on the Correlation Between Ψ_{stem} and $\delta^{13}C$

The effect of sampling granularity on the regression estimates of the relations between Ψ_{stem} and $\delta^{13}C$ of musts was assessed within the same producing Cabernet Sauvignon vineyard where the management zone clustering trial was conducted (section "Comparing $\delta^{13}C$ and Plant Water Status to Delineate Management Zones for Selective Harvest in Precision Viticulture"). The method is described "Materials and Methods–Statistical Analysis" and results are presented

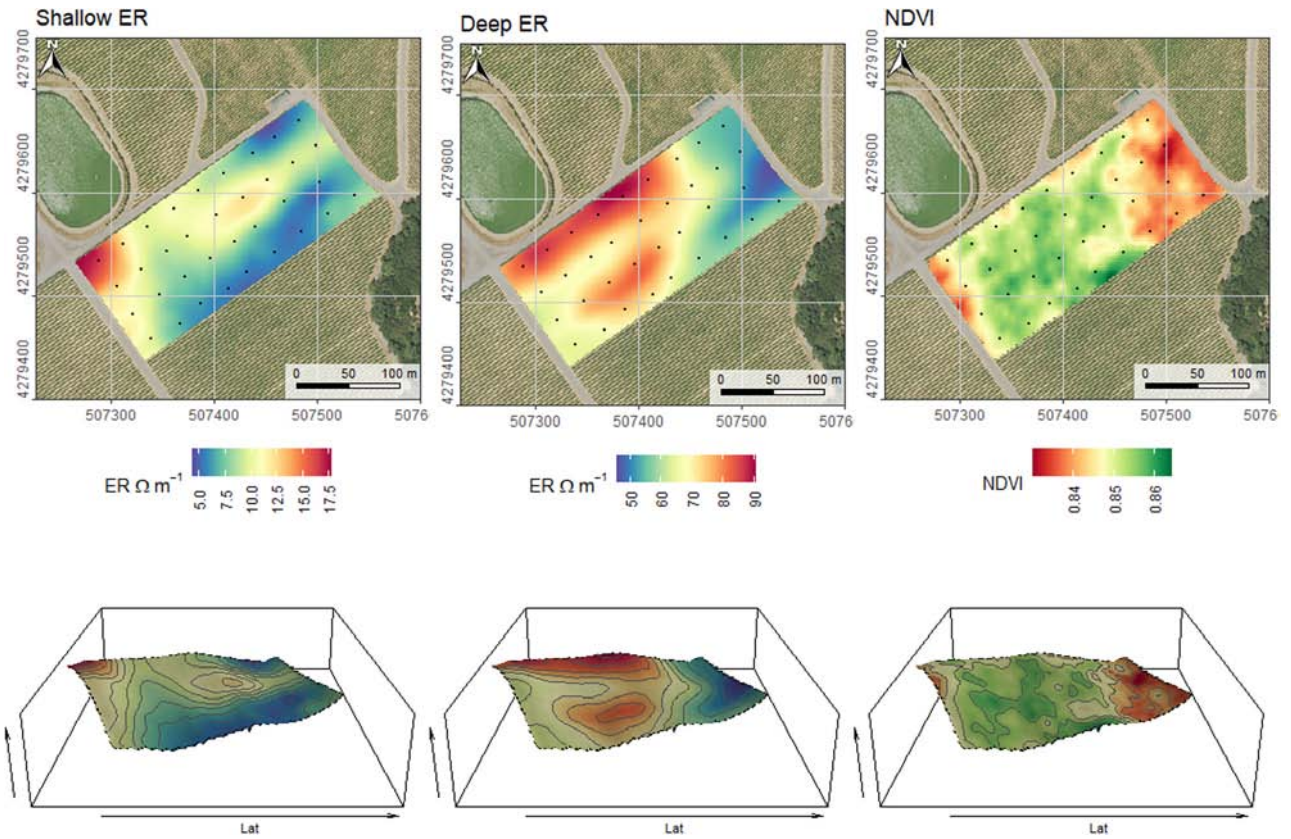

FIGURE 6 | Maps of shallow (0–0.75 m) and deep (0–1.5 m) soil electrical resistivity and canopy reflectance as NDVI. Black dots in 2D maps shown in the upper panels indicate the locations of the experimental units. Elevation is exaggerated twice in 3D maps to enhance topographic variation within the vineyard. Coordinates are EPSG:32610 (metric).

TABLE 3 | Spatial correlations (modified *t*-test) between terrain characteristics, soil electrical conductivity, ground sensed NDVI data and integrals of physiological measurement of plant water potential and leaf gas exchange.

	Slope	Elevation (m)	SAGA WI	TWI	Shallow ER	Deep ER	NDVI	Wood (kg)	SWP Int	$\delta^{13}C$	g_s Int	A_N Int	WUEi Int
NDVI	0.54	0.43	0.36	0.48*	0.03	0.59		0.60*	−0.29	0.03	−0.04	−0.12	0.05
Ψ_{stem} Int	−0.27	−0.78*	−0.57*	−0.07	−0.70**	−0.63*	−0.29	0.23		0.82***	−0.83***	−0.86***	−0.79***
$\delta^{13}C$	−0.07	0.46	0.37	−0.05	0.56*	0.42	−0.03	−0.52*	0.82***		−0.92***	−0.89***	0.92***
g_s Int	−0.05	−0.52	−0.40	−0.03	−0.62**	−0.36	−0.04	−0.51*	0.83***	−0.92***		0.97***	−0.92***
A_N Int	−0.09	−0.57*	−0.43	−0.07	−0.61**	−0.38	−0.12	0.39	0.86***	−0.89***	0.97***		−0.85***
WUEi Int	0.09	0.52	0.44	0.01	0.57*	0.48	0.05	−0.51*	−0.79***	0.92***	−0.92***	−0.85***	

*Column level Bonferroni significance threshold for alpha = 0.05 is 0.008. Stars indicate adjusted p-values, * < 0.05; ** < 0.01; *** < 0.001.*

in **Figure 10**. The purpose of this analysis was not to indicate a specific minimum number of measurements, but instead to indicate how low-quality results could be related to an insufficient sampling frequency of physiological data when compared to $\delta^{13}C$, which is an integrative and continuous indicator.

With the increasing number of measurement dates, the relationship between Ψ_{stem} and $\delta^{13}C$ was stronger, low R^2s were no longer estimated. The use of the minimum was able to produce the strongest relationship between variables but was the most sensitive to the number of measurements, showing the larger reduction in variability, and the larger increase in R^2 with increasing frequency. This is to be expected, as by increasing the number of measurements also increases the chance of finding the same minimum value across the resample combinations. For the same reason, the minimum also had generally higher variability in R^2 with respect to the use of mean or integral, except for a high number of measurements. The minimum also had lower variability in the slope with respect to the other aggregation values. A change in 1 unit of $\delta^{13}C$ corresponds to a variation in 0.2 MPa of minimum Ψ_{stem}. The mean and the integral behaved very similarly, within the confines of our work, no clear preference for one or the other statistics could be suggested.

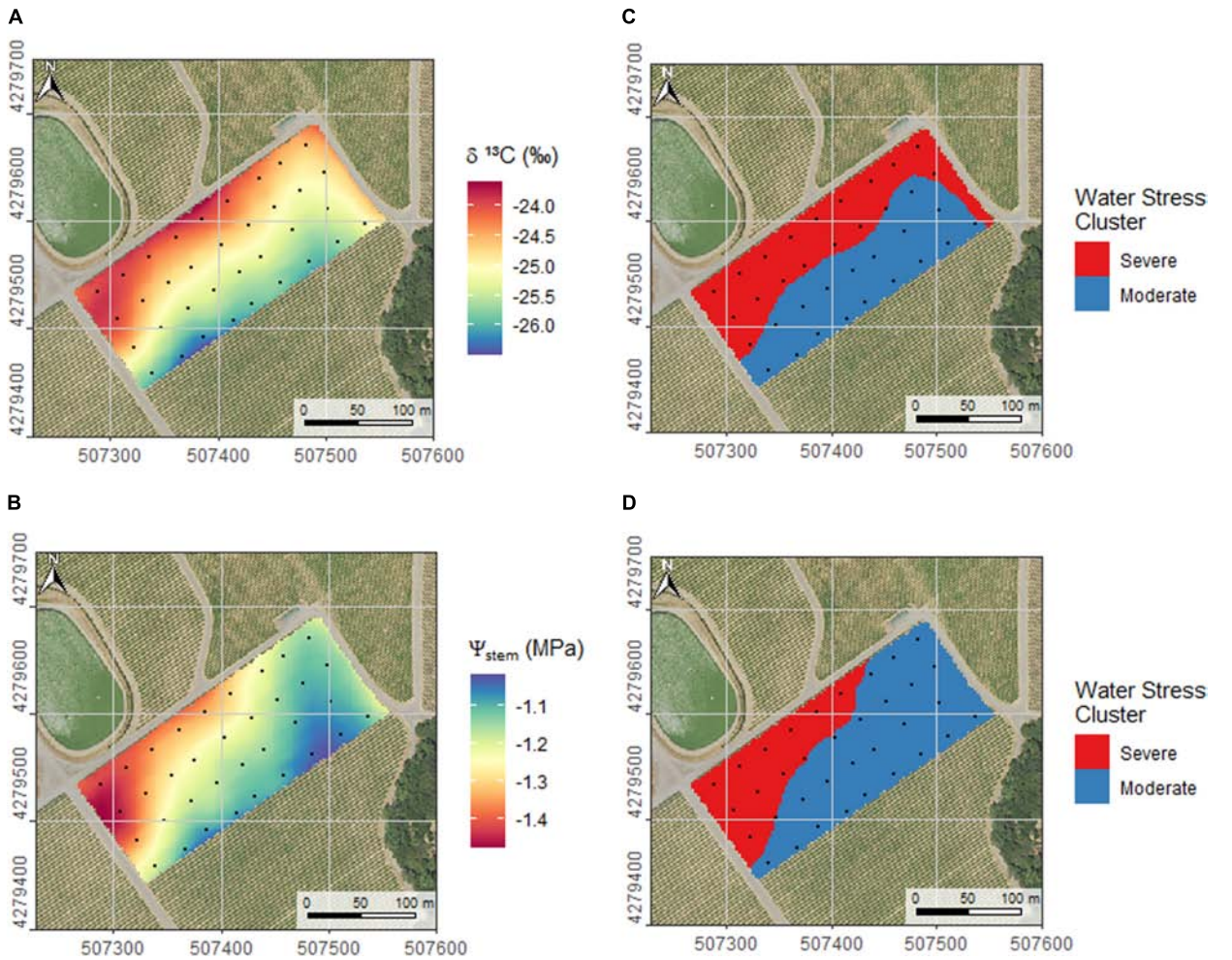

FIGURE 7 | Interpolated maps of $\delta^{13}C$ **(A)** and season integrals of Ψ_{stem} **(B)**. Maps where interpolated using a linear interpolation with *x*, *y* and *z* as ancillary variables. Root mean squared error in the bootstrap validation analysis is 0.63‰ with R^2 = 0.54 for interpolation of $\delta^{13}C$ and 0.1 MPa with R^2 = 0.56 for interpolation of Ψ_{stem}. K-means clustering of the field in two management zones for differential harvest according to water status throughout the season, as evaluated by $\delta^{13}C$ (**C**, clustering of data in **A**) and Ψ_{stem} integrals (**D**, clustering of data in **B**). Similarity between the clusters in **(C)** and **(D)** as expressed by Rand index is 0.67. Coordinates are EPSG:32610 (metric).

DISCUSSION

Carbon Isotope Composition of Grape Musts Is a Sensitive Bio-Data Logger of Plant Water Status and Gas-Exchange During Sugar Accumulation in Berries

Determining the relationship between Ψ and leaf gas exchange parameters is necessary for physiological and agricultural interpretation of $\delta^{13}C$. The significant and direct relationships presented here (**Figure 3**) provide evidence that $\delta^{13}C$ of grape musts was a sensitive bio-data logger of plant water status through berry development, as previously observed by other authors (Gaudillère et al., 2002; de Souza et al., 2005; Koundouras et al., 2008; Costantini et al., 2010; Brillante et al., 2018b). However, some contradictory results were also observed when $\delta^{13}C$ was measured on leaves (Poni et al., 2009; Bchir et al., 2016). To our knowledge, our study is the first work where a relationship of $\delta^{13}C$ of grape musts with A_N and g_s is presented, and between $\delta^{13}C$ and WUEi with such a large and diverse dataset (**Figure 4**). Our results are corroborated by those obtained in different crops such as avocado (*Persea Americana* Mill., Acosta-Rangel et al., 2018), peach (*Prunus persica*, L., Pascual et al., 2016), and rice (*Oryza sativa,* L., Tao et al., 2015).

It should be kept in mind that $\delta^{13}C$ signal is interpreted as a continuous integrator of the photosynthetic process, recording every moment of activity, while typical Ψ and leaf gas exchange determinations are discrete measurements (*i.e.,* performed at a specific time of the day and in few time points over the growing season). Besides, when measured on sink organs such as fruits, $\delta^{13}C$ integrates the carbon fixation processes at the scale of the whole canopy, while typical Ψ and leaf gas exchange measurements are performed on specific and selected leaves assumed representative of the whole. The differences in spatial

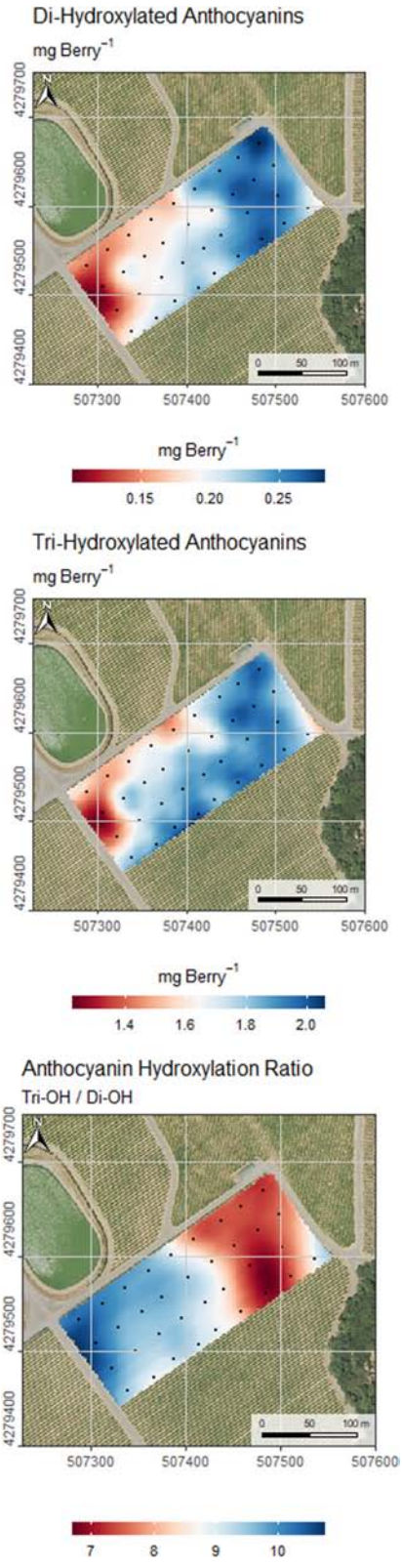

FIGURE 8 | Kriged maps of di-hydroxylated, and tri-hydroxylated anthocyanins expressed in mg berry^{-1} and hydroxylation ratio (tri-hydroxylated/di-hydroxylated). Coordinates are EPSG:32610 (metric).

and temporal scales of the variables in the correlations may affect the quality of the estimations. In **Figure 10**, we show how variability in regression estimates of the $\delta^{13}C \sim$ plant physiology measurements at discrete time points are affected by sampling granularity (i.e., the number of time points and summarizing function) of the discrete measurement. Not only the statistical significance and explained variability may be affected by the sampling granularity, but also slope and intercept may change. In the literature, following the seminal paper by Gaudillère et al. (2002), the use of minimum Ψ, instead than the average or the integral is frequently found to establish relationships with $\delta^{13}C$ (Guix-Hébrard et al., 2007; Brillante et al., 2016b). The minimum Ψ ranks the worst water status conditions across the experimental units in the dataset, the correlation with $\delta^{13}C$ appears more stable at a lower number of measurements (**Figure 10**) and the slope $1‰ \approx -0.2$ MPa confirm the results of a previous meta-analysis (Brillante et al., 2018b) which was, however, based on minimum Ψ_{pd} (predawn). This correspondence $\delta^{13}C \sim \Psi$ will easily allow direct interpretation of relative differences in $\delta^{13}C$, within and between vineyards, as absolute correspondence is not possible considering the intercept of the relationship appears to change because of environmental and genetic factors (Brillante et al., 2018b).

We would explicit that the statistical exercise shown in **Figure 10** does not have the aim of identifying the minimum number of measurements to achieve reliable correlations with $\delta^{13}C$ but to demonstrate that inconsistent results could be due to a reduced dataset for a fine integration of plant water status. We will not suggest a precise number of measurements needed for the regression analysis because this would be very dependent on the conditions of the study, as a result of the influence of weather conditions (especially vapor pressure deficit) at the time of the measurement on Ψ_{stem}, as observed in grapevine and other crops (Williams and Baeza, 2007; Suter et al., 2019). In the steady meteorological conditions of California summers, a relatively low number of measurements allows reliable correlations; in more variable weather conditions, this number could be higher. For instance, in Burgundy (Eastern France), weekly measurements of Ψ_{stem} were not related to $\delta^{13}C$, until a modeling approach allowed to compare averages from daily interpolations (Brillante et al., 2016b). In the same conditions, weekly measurements were enough to correlate $\delta^{13}C$ to Ψ_{pd} (Brillante et al., 2017), as the time of the day makes it less dependent on atmospheric conditions.

Genetic and Environmental Factors and the Role of Nitrogen

In this study, we pooled together three different varieties and maintained good correlations between $\delta^{13}C$ and other physiological measurements (**Figures 3–4**), but genetic variability in $\delta^{13}C$ was reported across grapevine cultivars (Gaudillère et al., 2002; Gómez-Alonso and García-Romero, 2009; Bota et al., 2016). This variability is worth investigating as a phenotyping tool for breeding purposes, as commonly done in other crops, but it would complicate the use of $\delta^{13}C$ in production management. The use of relative comparison as a simple turnaround has been suggested in the previous Section "Carbon Isotope Composition

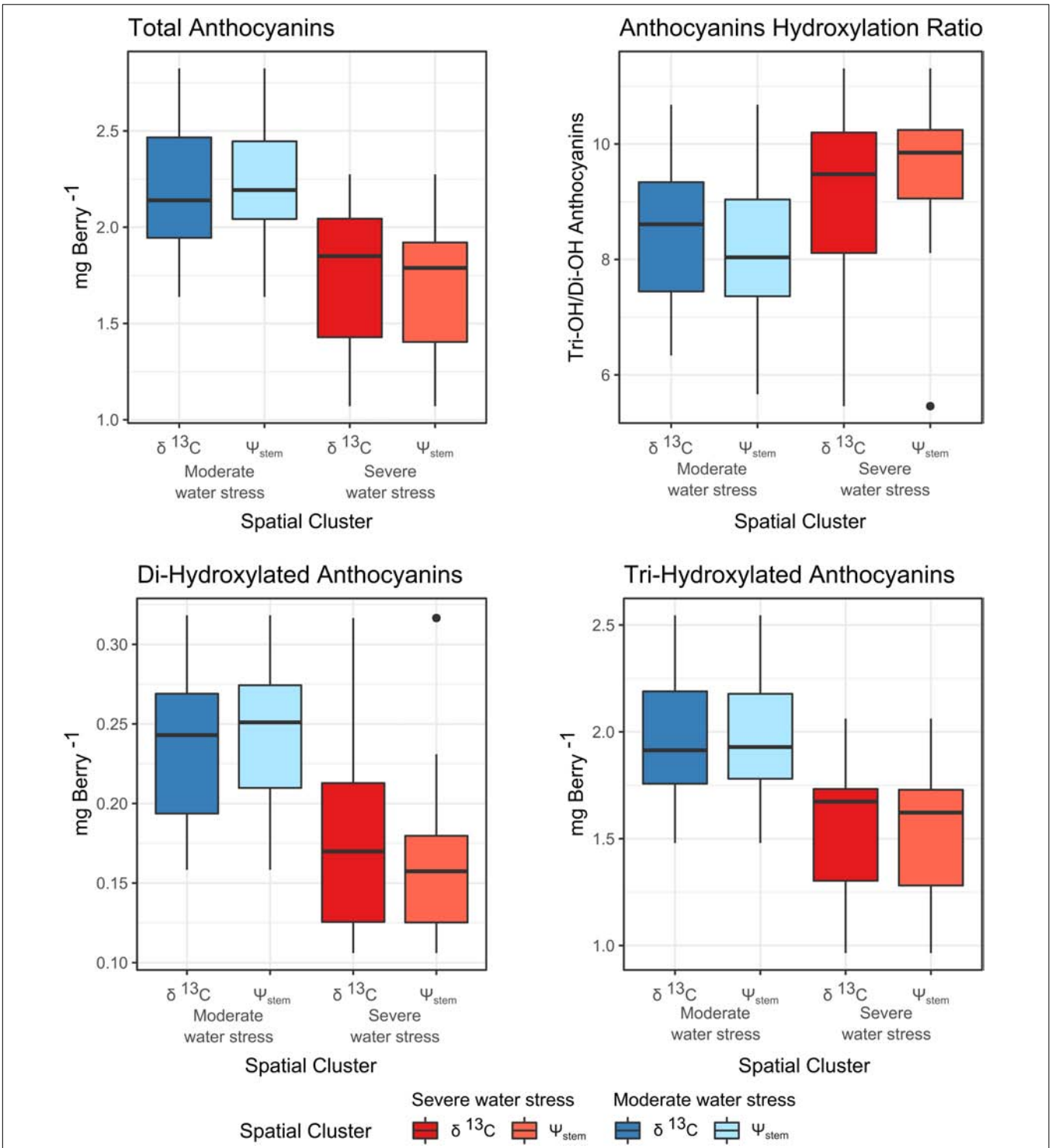

FIGURE 9 | Separation of mean anthocyanin composition (mg berry^{-1}) across the management zones presented in **Figures 7C,D**. Metabolite data from experimental units located within each zone were aggregated.

of Grape Musts Is a Sensitive Bio-Data Logger of Plant Water Status and Gas-Exchange During Sugar Accumulation in Berries." The use of $\delta^{13}C$ as a phenotyping tool would be very valuable in grapevine as well, considering the strong relationship with WUEi and gas exchange observed in **Figure 4**. However, several biological and environmental factors could contribute to differences in $\delta^{13}C$ between genotypes, including stomatal behavior (Miner et al., 2017), leaf structure and anatomy

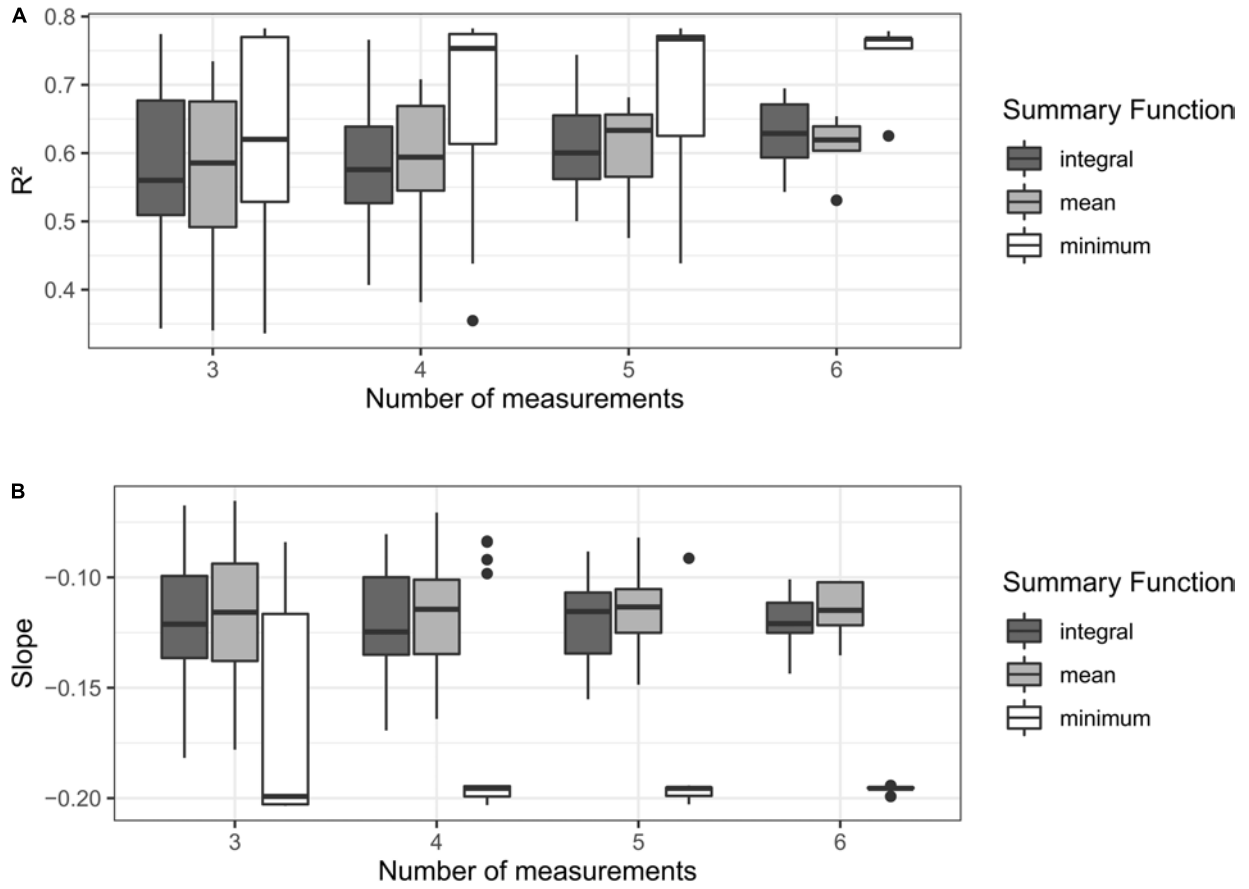

FIGURE 10 | Evolution of the linear regression estimates between Ψ_{stem} and $\delta^{13}C$ of musts at harvest (‰) in a simulation where measurement dates through the season for Ψ_{stem} is progressively increased. Sensitivity of different aggregation statistics for Ψ_{stem} is also tested: minimum, mean, and integral. In the linear regression $\Psi_{stem} = m\delta^{13}C + b$ **(A)** shows effect on R^2 and **(B)** shows effect on slope (m).

(Flexas et al., 2010), and factors influencing the C_i/C_a ratio, i.e. affecting A_N (Cernusak et al., 2013). In C_3 species, the dependency of carbon isotope discrimination on g_m has been deemed the cause of the relationships between $\delta^{13}C$ in leaves and A_N (von Caemmerer et al., 2014) which was also observed here (**Figure 4**). In our case, the correlation was obtained by measuring $\delta^{13}C$ in sink organs, thus after partitioning, and not instantaneously during photosynthesis.

Comparisons of results across space and time may be complicated by genotype x environment interactions, but also by environmental conditions affecting the $^{13}C/^{12}C$ ratio in the atmosphere (e.g., latitude, altitude, etc.). Following Gaudillère et al., 2002, in grapevine literature we traditionally use $\delta^{13}C$, thus referring values to a chemical standard only, but the use of carbon isotope discrimination, Δ (Brook et al., 2020), thus accounting for ^{13}C in the atmosphere, may be preferable. This approach would make data more comparable across time, considering that atmosphere is consistently enriching in ^{12}C as a result of fossil combustion, exchange with methane, etc., and could reduce the variability in the intercept observed in Brillante et al. (2018b).

Environmental factors and interactions with horticultural practices also can play a role in variability between cultivars and complicate interpretation of results across vineyards and regions. For example, mineral nutrition affects carbon fixation rates influencing demand for CO_2 in the mesophyll, C_i/C_a, and therefore, Δ (Cernusak et al., 2013). Across all mineral elements, nitrogen is the one more likely creating this effect, as shown in other woody plants such as *Quercus robur* L., *Pinus pinaster* Ait. (Guehl et al., 1995), or *Ficus insipida* Willd (Cernusak et al., 2007, 2013). To our knowledge, this is the first time that similar confirming results are reported in a cultivated plant such as grapevine (**Figure 5**). In previous work on the grapevine, where the effect of N on $\delta^{13}C$ was also investigated (Gaudillère et al., 2002), the range of leaf N was too limited to observe any influence. In our study, a much wider range of variation was obtained by grouping data from three vineyards and two varieties; such variability is likely much harder to find in a single vineyard, in the absence of drastic variability in soil available N.

The discussion of our results in **Figure 5** should consider that they were obtained under commercial production conditions and in concomitance with drought, which is likely to interact with N content, and constitute big difference respect to the previously cited works by Guehl et al. (1995) and Cernusak et al. (2007, 2013). Under the confines of our study, the correlation

between C_i/C_a and N reported herein could be related to a direct increase in A_N, with water stress factors also participating, as a correlation between ψ and N was observed. In principle, limited water supply is typically associated with reduced leaf expansion and lower transpiration resulting in higher leaf N content (Farquhar et al., 2002). Increased N content per leaf area has been reported as acclimation to optimize N economy under drought in willow (*Salix* spp.) (Weih et al., 2011), and to vary naturally in field conditions. Besides, species living in low-rainfall regions may have a greater amount of leaf N respect to species living in high rainfall environments (Wright et al., 2003). Our results corroborate previous reports in this sense, as the smallest grapevine canopies were found in water-stressed plants (as estimated by the correlation between pruned wood weights with WUEi, g_s and A_N in Sonoma vineyard, **Table 3**) having higher water use efficiency and leaf nitrogen content, while nitrogen was not correlated to the weight of dormant pruning (not shown). Our results are also in agreement with a recent meta-analysis (He and Dijkstra, 2014), indicating that availability of water, rather than N availability, could be the main driver for reduced plant growth with long-term water deficits. Future studies need to address and elucidate the inter-relationship between leaf nitrogen and carbon discrimination, across different water deficits. Although some variability in $\delta^{13}C$ associated with N content cannot be excluded, it is unlikely that the effect of N could be so strong to prevent a direct interpretation of $\delta^{13}C$ in terms of plant water stress, as the results of our article show. This consideration is reinforced by considering that the effect of water deficits on $\delta^{13}C$, and the effect of leaf N on $\delta^{13}C$ act in the same direction, and therefore the effect of water deficits on leaf N could indirectly reinforce the signal on $\delta^{13}C$.

The Use of $\delta^{13}C$ for Zoning Vineyard Variability

The variability in plant water status may influence the yield (Guilpart et al., 2014) and berry chemistry of fleshy fruits, such as grapes (Brillante et al., 2017), and originate spatial variability in agriculture performances between and within plots, as confirmed here. Although in some rain-fed premium wine regions, with a long cultivation history, this may be exploited for site-specific management purposes or to distinguish agricultural products, i.e., as one part of the *terroir* effect (van Leeuwen et al., 2004), in the majority of vineyards, and agricultural systems, under irrigation, this variability may be considered a problem to deal with. Having a reliable, cost and time-effective measurement of plant water status, such as the $\delta^{13}C$, is crucial in managing variability, across different spatial scales, from the field to the region, and for large surface modeling purposes of plant performances, such as yield (Santesteban et al., 2016). The use of $\delta^{13}C$ to map an overall estimation of plant water status was already proposed (Herrero-Langreo et al., 2013). However, the efficiency of zoning fruit composition, compared to maps obtained through water potential measurements (Brillante et al., 2017; Gaudin et al., 2017) was never evaluated. We provide evidence that the two strategies are equivalent in their performances of delineating vineyard management zones having different anthocyanin composition (**Figure 9**), useful for implementing a selective harvest approach (Bramley et al., 2011). However, it is the opinion of the authors that evaluation of plant water status by direct measurement and kriging is inefficient and costly to be implemented in commercial agriculture because of the numbers of measurement points needed in space and time for a successful interpolation and estimation of vineyard conditions. The use of sensors, such as electrical resistivity or NDVI as in here, to assess variability in vineyards is straightforward and inexpensive but requires a rigorous ground-truthing to provide reliable information that could be used for informed management. For example in our case, $\delta^{13}C$ was correlated to shallow ER, but not to deep ER or NDVI (**Table 3**). Measurement of $\delta^{13}C$ in grapes is reliable and effective for this purpose, especially considering that physical limits and physiological differences between management zones are influenced by the weather of the season and as such can change across years demanding for yearly checking. Vineyard variability can be easily mapped through the use of sensors, and physiological differences between management zones obtained in this way could be assessed on a single composite berry sample for each zone. A difference in $\delta^{13}C$ by 1‰ between the zones, corresponding to an average difference of 0.2 MPa in ψ_{stem} during the ripening period, would be enough to produce sensible effects on the grape composition, as in the case reported here.

CONCLUSION

Measuring carbon isotope discrimination of grape juice is fast and effective, needs to be performed only once a year for a comprehensive and reliable assessment of plant water status during the whole ripening season. The measurement is carried out on the same substratum routinely sampled to assess grape ripening and requires little additional processing. As demonstrated in this article, the relationships with grapevine gas exchange and water potentials are very robust. Noisy factors such as differences in regions, varieties, or leaf nitrogen are minimized and could be neglected when using $\delta^{13}C$ to map variability at the within vineyard scale, but may need to be considered if the variability is expected to be large, which also depends on the space-time scale. One solution would be comparing relative and not absolute differences in $\delta^{13}C$ values.

Carbon isotope discrimination is a continuous assessor and as such provides a more comprehensive estimate of plant physiology respect to discrete measurements obtained with other instruments or techniques allowing discrete measurements (Ψ, gas exchange). This is even more important when daily environmental variability is high. As shown here, the difference in sample granularity may affect the relationship with the discrete measurements and bring to conflicting results when the discrete measurement is aggregated from a small number of time points that do not consent a good assessment of the temporal variability.

The ability to provide reliable estimates of plant average conditions from a single measurement is an advantage of the $\delta^{13}C$ for zoning, thus in precision viticulture, and a limit for irrigation scheduling. With the increasing availability of sensor data to

monitor plant performances in agriculture fields, we need fast and reliable ground-truthing able to characterize plant physiology efficiently with a large scale and high resolution. This article demonstrated that $\delta^{13}C$ of grape must is a reliable and repeatable assessor of grapevine water status and gas exchange in vineyard systems that are crucial for zoning vineyards, independently if irrigated, and for rapid validation of sensor maps in precision viticulture from a physiological standpoint.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

LB designed the trial, analyzed the data and wrote the first version of the manuscript. SK acquired the funding. All authors executed the trial and contributed to the final version and approved it.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2020.561477/full#supplementary-material

FIGURE S1 | Relationship between stem water potential and leaf gas exchange in grapevine across three different vineyards in three different viticulture areas and two different varieties (Merlot and Cabernet-Sauvignon). Each point is an aggregate of minimum 3 measurements on different leaves and plants within a single experimental unit. **(a)** relationship with stomatal conductance; (g_s) **(b)** relationship with A_N; **(c)** relationship with intrinsic water use efficiency (WUEi). Lines are linear regression line and color is mapped to cultivar; in gray is the confidence interval.

FIGURE S2 | Relationship between $\delta^{13}C$ (‰) of grape musts at harvest and means of leaf gas exchange across three different vineyards in three different viticulture areas and two different varieties (Merlot and Cabernet-Sauvignon). **(a)** relationship with stomatal conductance (g_s), **(b)** 4 relationship with A_N; **(c)** relationship with intrinsic water use efficiency (WUEi). Blue line is a linear regression line; in gray is the confidence interval.

REFERENCES

Acevedo-Opazo, C., Tisseyre, B., Guillaume, S., and Ojeda, H. (2008). The potential of high spatial resolution information to define within-vineyard zones related to vine water status. *Prec. Agric.* 9, 285–302. doi: 10.1007/s11119-008-9073-1

Acosta-Rangel, A., Ávila-Lovera, E., De Guzman, M. E., Torres, L., Haro, R., Lu, M., et al. (2018). Evaluation of leaf carbon isotopes and functional traits in avocado reveals water-use effi cient cultivars. *Agric. Ecosyst. Environ.* 263, 60–66. doi: 10.1016/j.agee.2018.04.021

Bchir, A., Escalona, J. M., Gallé, A., Hernández-montes, E., Tortosa, I., Braham, M., et al. (2016). Carbon isotope discrimination (δ13C) as an indicator of vine water status and water use efficiency (WUE): looking for the most representative sample and sampling time. *Agric. Water Manag.* 167, 11–20. doi: 10.1016/j.agwat.2015.12.018

Bonfante, A., Monaco, E., Langella, G., Mercogliano, P., Bucchignani, E., Manna, P., et al. (2018). A dynamic viticultural zoning to explore the resilience of terroir concept under climate change. *Sci. Total Environ.* 624, 294–308. doi: 10.1016/j.scitotenv.2017.12.035

Bota, J., Tomás, M., Flexas, J., Medrano, H., and Escalona, J. M. (2016). Differences among grapevine cultivars in their stomatal behavior and water use efficiency under progressive water stress. *Agric. Water Manag.* 164, 91–99. doi: 10.1016/j.agwat.2015.07.016

Bramley, R. G. V., Ouzman, J., and Thornton, C. (2011). Selective harvesting is a feasible and profitable strategy even when grape and wine production is geared towards large fermentation volumes. *Aust. J. Grape Wine Res.* 17, 298–305. doi: 10.1111/j.1755-0238.2011.00151.x

Brillante, L., Bois, B., Lévêque, J., and Mathieu, O. (2016a). Variations in soil-water use by grapevine according to plant water status and soil physical-chemical characteristics-A 3D spatio-temporal analysis. *Eur. J. Agron.* 77, 122–135. doi: 10.1016/j.eja.2016.04.004

Brillante, L., Martínez-Lüscher, J., and Kurtural, S. K. (2018a). Applied water and mechanical canopy management affect berry and wine phenolic and aroma composition of grapevine (Vitis vinifera L., *cv. Syrah*) in central California. *Sci. Hortic.* 227, 261–271. doi: 10.1016/j.scienta.2017.09.048

Brillante, L., Martínez-Luscher, J., Yu, R., Plank, C. M., Sanchez, L., Bates, T. L., et al. (2017). Assessing spatial variability of grape skin flavonoids at the vineyard scale based on plant water status mapping. *J. Agric. Food Chem.* 65, 5255–5265. doi: 10.1021/acs.jafc.7b01749

Brillante, L., Mathieu, O., Lévêque, J., and Bois, B. (2016b). Ecophysiological modeling of grapevine water stress in burgundy terroirs by a machine-learning approach. *Front. Plant Sci.* 7:796. doi: 10.3389/fpls.2016.00796

Brillante, L., Mathieu, O., Lévêque, J., van Leeuwen, C., and Bois, B. (2018b). Water status and must composition in grapevine cv. Chardonnay with different soils and topography and a mini meta-analysis of the δ13C/water potentials correlation. *J. Sci. Food Agric.* 98, 691–697. doi: 10.1002/jsfa.8516

Brook, A., De Micco, V., Battipaglia, G., Erbaggio, A., Ludeno, G., Catapano, I., et al. (2020). A smart multiple spatial and temporal resolution system to support precision agriculture from satellite images: proof of concept on *Aglianico vineyard. Remote Sens. Environ.* 240:111679. doi: 10.1016/j.rse.2020.111679

Bustan, A., Dag, A., Yermiyahu, U., Erel, R., Presnov, E., Agam, N., et al. (2016). Fruit load governs transpiration of olive trees. *Tree Physiol.* 36, 380–391. doi: 10.1093/treephys/tpv138

Castellarin, S. D., Matthews, M. A., Di Gaspero, G., and Gambetta, G. A. (2007). Water deficits accelerate ripening and induce changes in gene expression regulating flavonoid biosynthesis in grape berries. *Planta* 227, 101–112. doi: 10.1007/s00425-007-0598-8

Cernusak, L. A., Ubierna, N., Winter, K., Holtum, J. A. M., Marshall, J. D., and Farquhar, G. D. (2013). Environmental and physiological determinants of carbon isotope discrimination in terrestrial plants. *New Phytol.* 200, 950–965. doi: 10.1111/nph.12423

Cernusak, L. A., Winter, K., Aranda, J., Turner, B. L., and Marshall, J. D. (2007). Transpiration efficiency of a tropical pioneer tree (*Ficus insipida*) in relation to soil fertility. *J. Exp. Bot.* 58, 3549–3566. doi: 10.1093/jxb/erm201

Chaves, M. M., Zarrouk, O., Francisco, R., Costa, J. M., Santos, T., Regalado, A. P., et al. (2010). Grapevine under deficit irrigation: hints from physiological and molecular data. *Ann. Bot.* 105, 661–676. doi: 10.1093/aob/mcq030

Conrad, O., Bechtel, B., Bock, M., Dietrich, H., Fischer, E., Gerlitz, L., et al. (2015). System for Automated Geoscientific Analyses (SAGA) v. 2.1.4. *Geosci. Model Dev.* 8, 1991–2007. doi: 10.5194/gmd-8-1991-2015

R Core Team (2018). *R:A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.

Costantini, E. A. C., Pellegrini, S., Bucelli, P., Barbetti, R., Campagnolo, S., Storchi, P., et al. (2010). Mapping suitability for Sangiovese wine by means of δ13C and geophysical sensors in soils with moderate salinity. *Eur. J. Agron.* 33, 208–217. doi: 10.1016/j.eja.2010.05.007

de Souza, C. R., Maroco, J. P., dos Santos, T. P., Rodrigues, M. L., Lopes, C. M., Pereira, J. S., et al. (2005). Impact of deficit irrigation on water use efficiency and carbon isotope composition (delta13C) of field-grown grapevines under Mediterranean climate. *J. Exp. Bot.* 56, 2163–2172. doi: 10.1093/jxb/eri216

Diffenbaugh, N. S., and Scherer, M. (2013). Using climate impacts indicators to evaluate climate model ensembles: temperature suitability of premium winegrape cultivation in the United States. *Clim. Dyn.* 40, 709–729. doi: 10.1007/s00382-012-1377-1

Dutilleul, P. (1993). Modifying the t test for assessing the correlation between two spatial processes. *Biometrics* 49, 305–314.

Farquhar, G. D., Buckley, T. N., and Miller, J. M. (2002). Optimal stomatal control in relation to leaf area and Nitrogen content. *Silva Fenn.* 36, 625–637.

Farquhar, G. D., Ehleringer, J. R., and Hubick, K. T. (1989). Carbon Isotope Discrimination and Photosynthesis. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 40, 503–537. doi: 10.1146/annurev.pp.40.060189.002443

Flexas, J., Galmés, J., Gallé, A., Gulias, J., Pou, A., Ribas-Carbo, M., et al. (2010). Improving water use efficiency in grapevines: potential physiological targets for biotechnological improvement. *Aust. J. Grape Wine Res.* 16, 106–121. doi: 10.1111/j.1755-0238.2009.00057.x

Gaudillère, J.-P., Van Leeuwen, C., and Ollat, N. (2002). Carbon isotope composition of sugars in grapevine, an integrated indicator of vineyard water status. *J. Exp. Bot.* 53, 757–763.

Gaudin, R., Roux, S., and Tisseyre, B. (2017). Linking the transpirable soil water content of a vineyard to predawn leaf water potential measurements. *Agric. Water Manag.* 182, 13–23. doi: 10.1016/j.agwat.2016.12.006

Gavlak, R. G., Horneck, D. A., and Miller, R. O. (1994). "Plant, soil and water reference methods for the western region," *Western Regional Extension Publication (WREP)* Fairbanks: University of Alaska, 125.

Gómez-Alonso, S., and García-Romero, E. (2009). Effect of irrigation and variety on oxygen (δ18O) and carbon (δ13C) stable isotope composition of grapes cultivated in a warm climate. *Aust. J. Grape Wine Res.* 16, 283–289. doi: 10.1111/j.1755-0238.2009.00089.x

Guehl, J.-M., Fort, C., and Ferhi, A. (1995). Differential response of leaf conductance, carbon isotope discrimination and water- use efficiency to nitrogen deficiency in maritime pine and pedunculate oak plants. *New Phytol.* 131, 149–157.

Guilpart, N., Metay, A., and Gary, C. (2014). Grapevine bud fertility and number of berries per bunch are determined by water and nitrogen stress around flowering in the previous year. *Eur. J. Agron.* 54, 9–20. doi: 10.1016/j.eja.2013.11.002

Guix-Hébrard, N., Voltz, M., Trambouze, W., Garnier, F., Gaudillère, J. P., and Lagacherie, P. (2007). Influence of watertable depths on the variation of grapevine water status at the landscape scale. *Eur. J. Agron.* 27, 187–196. doi: 10.1016/j.eja.2007.03.005

Gutiérrez, S., Diago, M. P., Fernández-Novales, J., and Tardaguila, J. (2018). Vineyard water status assessment using on-the-go thermal imaging and machine learning. *PLoS One* 13:e0192037. doi: 10.1371/journal.pone.0192037

He, M., and Dijkstra, F. A. (2014). Drought effect on plant nitrogen and phosphorus?: a meta- analysis. *New Phytol.* 204, 924–931.

Herrero-Langreo, A., Tisseyre, B., Goutouly, J.-P. P., Scholasch, T., and Van Leeuwen, C. (2013). Mapping grapevine (*Vitis vinifera* L.) water status during the season using carbon isotope ratio (δ13C) as ancillary data. *Am. J. Enol. Vitic.* 64, 307–315. doi: 10.5344/ajev.2013.12125

Hoekstra, A. Y., and Mekonnen, M. M. (2012). The water footprint of humanity. *Proc. Natl. Acad. Sci. U.S.A.* 109, 3232–3237. doi: 10.1073/pnas.1109936109

Koundouras, S., Tsialtas, I. T., Zioziou, E., and Nikolaou, N. (2008). Rootstock effects on the adaptive strategies of grapevine (*Vitis vinifera* L. cv. Cabernet-Sauvignon) under contrasting water status: Leaf physiological and structural responses. *Agric. Ecosyst. Environ.* 128, 86–96. doi: 10.1016/j.agee.2008.05.006

Kustas, W. P., Agam, N., and Ortega-Farias, S. (2019). Forward to the GRAPEX special issue. *Irrig. Sci* 37, 221–226. doi: 10.1007/s00271-019-00633-7

Martínez-Lüscher, J., Brillante, L., and Kurtural, S. K. (2019). Flavonol profile is a reliable indicator to assess canopy architecture and the exposure of red wine grapes to solar radiation. *Front. Plant Sci.* 10:10. doi: 10.3389/fpls.2019.00010

Martínez-Lüscher, J., Brillante, L., Nelson, C. C. C., Al-Kereamy, A. M. A. M., Zhuang, S., and Kurtural, S. K. K. (2017). Precipitation before bud break and irrigation affect the response of grapevine 'Zinfandel' yields and berry skin phenolic composition to training systems. *Sci. Hortic.* 222, 153–161. doi: 10.1016/j.scienta.2017.05.011

Maupin, M. A., Kenny, J. F., Hutson, S. S., Lovelace, J. K., Barber, N. L., and Linsey, K. S. (2014). Estimated use of water in the United States in 2010: U.S. Geological Survey Circular 1405, 56. doi: 10.3133/cir1405

Medrano, H., Tomás, M., Martorell, S., Escalona, J.-M., Pou, A., Fuentes, S., et al. (2015). Improving water use efficiency of vineyards in semi-arid regions. *Rev. Agron. Sustain. Dev.* 35, 499–517. doi: 10.1007/s13593-014-0280-z

Miner, G. L., Bauerle, W. L., and Baldocchi, D. D. (2017). Estimating the sensitivity of stomatal conductance to photosynthesis: a review. *Plant Cell Environ.* 40, 1214–1238. doi: 10.1111/pce.12871

Myers, N., Mittermeler, R. A., Mittermeler, C. G., Da Fonseca, G. A. B., and Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature* 403, 853–858. doi: 10.1038/35002501

Pascual, M., Villar, J. M., and Rufat, J. (2016). Water use efficiency in peach trees over a four-years experiment on the effects of irrigation and nitrogen application. *Agric. Water Manag.* 164, 253–266. doi: 10.1016/j.agwat.2015.10.021

Pebesma, E. J. (2004). Multivariable geostatistics in S: the gstat package. *Comput. Geosci.* 30, 683–691. doi: 10.1016/j.cageo.2004.03.012

Poni, S., Bernizzoni, F., Civardi, S., Gatti, M., Porro, D., and Camin, F. (2009). Performance and water-use efficiency (single-leaf vs. whole-canopy) of well-watered and half-stressed split-root *Lambrusco grapevines* grown in Po Valley (Italy). *Agric. Ecosyst. Environ.* 129, 97–106. doi: 10.1016/j.agee.2008.07.009

Ritchey, J. G., and Andrew, L. A. (1999). A standard red wine: monomeric analysis of commercial Cabernet-Sauvignon wines. *Am. J. Enol. Vitic.* 50, 91–100.

Sanchez, L. A., Sams, B., Alsina, M. M., Hinds, N., Klein, L. J., and Dokoozlian, N. (2017). Improving vineyard water use efficiency and yield with variable rate irrigation in California. *Adv. Anim. Biosci.* 8, 574–577. doi: 10.1017/s2040470017000772

Santesteban, L. G., Miranda, C., and Royo, J. B. (2016). Interest of carbon isotope ratio (δ13C) as a modeling tool of grapevine yield, berry size and sugar content at within-field, winegrowing domain and regional scale. *Theor. Exp. Plant Physiol.* 28, 193–203. doi: 10.1007/s40626-016-0067-5

Suter, B., Triolo, R., Pernet, D., Dai, Z., and Van Leeuwen, C. (2019). Modeling stem water potential by separating the effects of soil water availability and climatic conditions on water status in grapevine (*Vitis vinifera* L.). *Front. Plant Sci.* 10:1485. doi: 10.3389/fpls.2019.01485

Tao, Y., Zhang, Y., Jin, X., Saiz, G., Jing, R., Guo, L., et al. (2015). More rice with less water – evaluation of yield and resource use efficiency in ground cover rice production system with transplanting. *Eur. J. Agron.* 68, 13–21. doi: 10.1016/j.eja.2015.04.002

Taylor, J. A., Acevedo-Opazo, C., Ojeda, H., and Tisseyre, B. (2010). Identification and significance of sources of spatial variation in grapevine water status. *Aust. J. Grape Wine Res.* 16, 218–226. doi: 10.1111/j.1755-0238.2009.00066.x

van Leeuwen, C., Friant, P., Choné, X., Tregoat, O., Koundouras, S., and Dubourdieu, D. (2004). Influence of climate, soil, and cultivar on terroir. *Am. J. Enol. Vitic.* 55, 207–217.

von Caemmerer, S., Ghannoum, O., Pengelly, J. J. L., and Cousins, A. B. (2014). Carbon isotope discrimination as a tool to explore C4 photosynthesis. *J. Exp. Bot.* 65, 3459–3470. doi: 10.1093/jxb/eru127

Weih, M., Bonosi, L., Ghelardini, L., and Rönnberg-Wästljung, A. C. (2011). Optimizing nitrogen economy under drought: increased leaf nitrogen is an acclimation to water stress in willow (Salix spp.). *Ann. Bot.* 108, 1347–1353. doi: 10.1093/aob/mcr227

White, M. A., Diffenbaugh, N. S., Jones, G. V., Pal, J. S., and Giorgi, F. (2006). Extreme heat reduces and shifts United States premium wine production in the 21st century. *Proc. Natl. Acad. Sci. U.S.A.* 103, 11217–11222. doi: 10.1073/pnas.0603230103

Williams, L. E., and Baeza, P. (2007). Relationships among ambient temperature and vapor pressure deficit and leaf and stem water potentials of fully irrigated, field-grown grapevines. *Am. J. Enol. Vitic.* 58, 173–181.

Wright, I. J., Reich, P. B., and Westoby, M. (2003). Least-cost input mixtures of water and nitrogen for photosynthesis. *Am. Nat.* 161, 98–111.

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OPINION
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Unbiased Scientific Approaches to the Study of *Terroir* Are Needed!

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THE CONCEPT OF TERROIR: USE AND ABUSE

Terroir is a French term with roots in the Latin term *terra* meaning ground or land. In the international wine jargon, the term has assumed a more specific and nuanced meaning: it is the result of "collective knowledge of the interactions" between the environment and the vines mediated through human action and "providing distinctive characteristics" to the final product (i.e., wine; OIV, 2010). *Terroir* is not just a geographical site, but includes:

(i) the physical environment (e.g., climate, geology, soil, and topography);
(ii) the biological material and production practices;
(iii) cultural, socio-economical and political aspects.

Nowadays, the storytelling of *terroir* is abundant in both the popular press and marketing of wine. Although there is no reference to wine quality in the description of the *terroir* concept (OIV, 2010), wines which may be associated with a single vineyard are often deemed superior, although wines derived from multiple sites may also be highly regarded (Bramley, 2017).

According to OIV, 2010, which should be considered an accepted definition, *terroir* is a loose interpretation of a protected designation of origin (PDO), thus questioning the need for a wine-specific term. For example, in the regulation of the European Union (EC No. 510/2006 Art. 2.1(a), Council of the European Union, 2006), in order to benefit from PDO status, an agricultural product needs to:

i) originate and be produced, processed and prepared in the defined geographical area and
ii) have "quality or characteristics essentially or exclusively due to a particular geographical environment with its inherent natural and human factors."

The concept of geographical origin can be used in all crops and foods, and PDOs are defined and regulated. Conversely, the *terroir* interpretation applies mostly to wine and is not regulated, which leaves it open to abuse and self-assessment without control, scientific evidence or socio-historical recognition (Matthews, 2016). As a result, in the current popular use, the term *terroir* has erroneously become jargon for vineyard site.

A designation of origin is a strict regulation. In order to maintain characteristics related to the place and the traditional practices, PDO products are made according to production standards, and are evaluated before introduction to the market, to ensure conformity to the important and distinctive characteristics that are the reason for the designation. It should be clear that, when

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including all aspects, a PDO is a product that brings a sense of place (although, PDO delineation most commonly follows political boundaries and not the limits derived from scientific understandings). Reverse engineering of geographically designated products may be straightforward, but it is definitely harder to recreate the collective knowledge, the human and cultural aspects, which are crucial to the past but also the future developments of food.

SCIENTIFIC APPROACHES

The predominant scientific focus of terroir research is on the relationships between plants and the environment, driven by site variability, and the effects of production methods, in interaction with the environment, on crop composition (van Leeuwen et al., 2004). Somewhat confusingly, in wine science, this specific branch that does not include cultural or socio-economical aspects, is also referred to using the term *terroir*. *Terroir de base* (Deloire et al., 2005), or less confusingly, a "functional zone" is the smallest area where it is possible to objectively describe the effect of environment on plant physiology and agricultural production, and which could be differentially managed (Bramley, 2005; Acevedo-Opazo et al., 2008; Bonfante et al., 2015; Brillante et al., 2016a; Brillante et al., 2017; Priori et al., 2019; Bramley, 2020). The approach to studying relationships between *terroir* and wine peculiarities is scale dependent, as different factors influence agricultural choices and vine performance at the regional or the within vineyard level (Vaudour, 2002). Recently, the science on the topic has shifted from a largely descriptive regional science to a technical research field, focused on the study of variation in the biophysical characteristics of the vineyard site (soil, climate, topography, etc.) and its interaction with vine performance; much of this work has involved precision agriculture methodologies (Bramley, 2020 and references therein). Currently, most of this biophysical research relating to *terroir* is based on zoning and the description of plant-environment interactions at a given site (Vaudour et al., 2015; White, 2020).

The science of describing site-specific relationships between the physical environment, the vine and the wine has a shorter life than the social and historical recognition of regions and vineyards. A better scientific understanding of the mechanisms ruling vineyard variability and grape quality (also with more fundamental biological approaches, e.g., Tramontini et al., 2013; Dal Santo et al., 2018) would definitely benefit all players in the industry, from producers to marketers and wine writers in both the "New" and the "Old World." We would expect the science to be a critical and proactive investigation, using current understanding of viticultural systems to provide directions for managing the quality of wines or suggesting more efficient viticultural practices; and not simply serving the role of justifying the status quo preferred by wine writers and wine marketers of iconic wines and regions.

The identification and mapping of agricultural sites with similar characteristics from a physical point of view, also called zoning, is the first step in defining a designated area and has crucial importance also in precision and sustainable agriculture, with positive economic implications. Furthermore, the availability of spatial-temporal data obtained through affordable and rapid sensing technologies has paved the path to an efficient delineation of within-field variability so that this scale of study has become more valuable to growers for management purposes. The adoption of precision viticulture and variable rate technologies is presently exploding, but issues persist in data synthesis from multiple and not always reliable sources, and with the practical implementation of information obtained (Vaudour et al., 2015).

Agricultural zoning procedures applied at different spatial scales enable site-specific planning and contribute to the optimization of management in vineyard agroecosystems. Site-specific management is expected to improve the efficiency of production (Bramley et al., 2011; Tardaguila et al., 2011; Bramley, 2020), while the optimization of agricultural decisions according to the site characteristics is likely to enhance the peculiarities of the product that depend on these characteristics.

There is no unique zoning method. Sometimes the quantitative link between the soil–plant-atmosphere system and wine only relies on empirical description, but instead, it should be analyzed with regard to its spatial structure and spatio-temporal patterns (Brillante et al., 2016a; Brillante et al., 2016b; Bramley, 2017; Brillante et al., 2017) and causal relationships (Bonfante et al., 2011; Bonfante et al., 2015; Bonfante et al., 2018). In other words, the *terroir* concept has often been treated and accepted as a "black box" or a marketing construct in which the relationships between a wine and its origin have not been clearly elucidated. There is a need for more and new multidisciplinary approaches in *terroir* analysis and zoning, to study the effects of soil, plant and climate systems on the characteristics of wines, and their resilience to stressors such as climate change. It is accepted that elements of what is needed have been researched in the context of improved viticultural management. But here our focus is on understanding terroir with the view that through such understanding, our ability to manage the production of wines of desired style will be enhanced (e.g., Bramley, 2017). Quantitative dynamic and spatial modeling approaches are likely an essential part of such research.

FUTURE DIRECTIONS

Reflecting the origin and production methods is a property of all wines. The whole industry will therefore benefit from an enhanced understanding of the mechanisms regulating the effects of the site on the wine free from dogmas and preconceptions based on our current assumptions. Knowledge that was often derived by the description of specific conditions in geographically limited places, and is likely hard to scale across space and time, especially given the current change in climate is no longer sufficient. For example, soil hydraulic properties are clearly an important element of *terroir* (Seguin, 1986; Choné et al., 2001; van Leeuwen et al., 2004; Bonfante et al., 2011; Bonfante et al., 2018), yet their importance relative to other

factors may depend on whether irrigation is a part of the production system as in dry areas where rainfall is not sufficient.

A key factor in the production of winegrapes is the conditions during ripening. This derives from the fact that the current notion of *terroir* effects originated from the historical viticultural areas of Europe, predominantly located at high latitudes and presenting limiting growing conditions because of relatively short growing seasons, rain and cold weather. In such environments, soil conditions that enhance ripening, independently of seasonal effects, facilitate consistent quality and have been associated with presence of water or nitrogen deficits (van Leeuwen and Seguin, 2006). Such stresses have been thought to enhance the composition of grapes primarily as a consequence of the acceleration in ripening, effects on secondary pathways (Castellarin et al., 2007a; Castellarin et al., 2007b) and also for the fact that they promote an ability to harvest fruit before any incidence of rot. Such considerations may be less important in other viticultural regions (e.g., predominantly dry areas such as Australia, California, and Southern Europe), where quality wine is nevertheless produced and recognized to be representative of its origins. There is much to understand about site-specific relationships and effects on grape composition beyond the role of variability in major drivers of plant physiology (water and nitrogen) and their effects on ripening status and kinetics; these should be investigated in future studies in both "Old World" and "New World" *terroirs*.

Plant water availability has for a long time being recognized as a major driver in the production of fine wines (Seguin, 1986), yet irrigated vineyards under uniform management have been shown to exhibit within-vineyard variations (e.g., Bramley et al., 2011; Brillante et al., 2017); that is, and given the associated demonstrated variation in vine vigor and fruit composition, the use of irrigation has not dulled the effect of the site. Similarly, all vineyards are fertilized in one way or another and higher nitrogen supply is considered necessary to high quality white wines (des Gachons et al., 2005), yet crop nutrition is rarely mentioned as a driver of *terroir* (Costantini et al., 2013; White, 2020). It is obvious that with excess nutrition and water, a large part of the environmental influence on plant physiology response may be eliminated, eventually flattening out a portion of the variability in grape composition between sites. Other cultural practices would have the same effect if drastically applied, but reasoned and moderate applications would not compromise differences between sites, even at the within-vineyard scale (Brillante et al., 2017).

We should not neglect that climate change will definitely impose a huge challenge on our current understandings and traditions, that will most likely also affect *terroirs*. In theory, the concept is dynamic, meaning that growers would adapt to changing conditions to preserve the unique character of their wine (van Leeuwen et al., 2013). However, it is not clear how the whole system will be affected, especially in heavily regulated viticulture areas where vineyards are ranked in a non-mutable way. Will these ranks, and the claimed superiority of specific sites withstand the effects of climate change? Not all aspects will be challenged in the same way. The core of the concept is independent from changes (site variability will always influence the final product although in different ways), but the social/historical recognition is not; will social appreciation change if varietal composition changes? For this same reason, it would be wise to abandon the idea of arbitrary climatic limits for the production of fine wines which express their place (van Leeuwen and Seguin, 2006), the validity of which has never been scientifically demonstrated.

The science of grapes and wines risks entering a seIf-referential bubble if it does not acknowledge that other crops are doing the same with different names. It is important to keep in mind that what really matters is the idea that variability in the environment affects agricultural production. This variability can be identified and managed to smooth differences when the goal is uniformity and consistency across vintages or to enhance differences when trying to produce distinctive wines. This is possible only through the development of fundamental, objective and unbiased understandings of vine-environment relationships and their application through precision agriculture techniques. That is, both wine research and wine production should rely on science rather than myths. Accordingly, communication of terroir to consumers should be based on evidence. Such an approach can only reinforce the concept of terroir and ensure its persistence and utility, even in instances where this relates primarily to the marketing of wines.

AUTHOR CONTRIBUTIONS

All authors contributed to the writing and reviewing of this manuscript.

REFERENCES

Acevedo-Opazo, C., Tisseyre, B., Guillaume, S., and Ojeda, H. (2008). The potential of high spatial resolution information to define within-vineyard zones related to vine water status. *Precis. Agric.* 9 (5), 285–302. doi:10.1007/s11119-008-9073-1

Bonfante, A., Agrillo, A., Albrizio, R., Basile, A., Buonomo, R., De Mascellis, R., et al. (2015). Functional homogeneous zones (fHZs) in viticultural zoning procedure: an Italian case study on Aglianico vine. *SOIL* 1, 427–441. doi:10.5194/soil-1-427-2015

Bonfante, A., Basile, A., Langella, G., Manna, P., and Terribile, F. (2011). A physically oriented approach to analysis and mapping of terroirs. *Geoderma* 167–168, 103–117. doi:10.1016/j.geoderma.2011.08.004

Bonfante, A., Monaco, E., Langella, G., Mercogliano, P., Bucchignani, E., Manna, P., et al. (2018). A dynamic viticultural zoning to explore the resilience of terroir concept under climate change. *Sci. Total Environ.* 624, 294–308. doi:10.1016/j.scitotenv.2017.12.035

Bramley, R. G. V. (2005). Understanding variability in winegrape production systems 2. Within vineyard variation in quality over several vintages. *Aust. J. Grape Wine Res.* 11, 33–42. doi:10.1111/j.1755-0238.2005.tb00277.x

Bramley, R. G. V. (2017). "Making sense of a sense of place," in Proceedings of the 16th Australian wine industry conference, adelaide, south Australia, Adelaide, South Australia, July 2016. Editors K. S. Beames, E. M. C. Robinson, P. R. Dry, and D. L. Johnson (Urrbrae, South Australia: Adelaide: The Australian Wine Industry Technical Conference Inc.), 45–51.

Bramley, R. G. V. (2020). "Precision viticulture: managing vineyard variability for improved quality outcomes," in *Managing wine quality: viticulture and wine quality*. New York, NY: Elsevier, Vol. 1. doi:10.1016/C2016-0-02279-9.

Bramley, R. G. V., Ouzman, J., and Boss, P. K. (2011). Variation in vine vigour, grape yield and vineyard soils and topography as indicators of variation in the chemical composition of grapes, wine and wine sensory attributes. *Aust. J. Grape Wine Res.* 17, 217–229. doi:10.1111/j.1755-0238.2011.00136.x

Brillante, L., Bois, B., Lévêque, J., and Mathieu, O. (2016a). Variations in soil-water use by grapevine according to plant water status and soil physical-chemical characteristics-A 3D spatio-temporal analysis. *Eur. J. Agron.* 77, 122–135. doi:10.1016/j.eja.2016.04.004

Brillante, L., Mathieu, O., Lévêque, J., and Bois, B. (2016b). Ecophysiological modeling of grapevine water stress in burgundy terroirs by a machine-learning approach. *Front. Plant Sci.* 7, 1–13. doi:10.3389/fpls.2016.00796

Brillante, L., Martínez-Luscher, J., Yu, R., Plank, C. M., Sanchez, L., Bates, T. L., et al. (2017). Assessing spatial variability of grape skin flavonoids at the vineyard scale based on plant water status mapping. *J. Agric. Food Chem.* 65, 5255–5265. doi:10.1021/acs.jafc.7b01749

Castellarin, S. D., Matthews, M. A., Di Gaspero, G., and Gambetta, G. A. (2007a). Water deficits accelerate ripening and induce changes in gene expression regulating flavonoid biosynthesis in grape berries. *Planta* 227, 101–112. doi:10.1007/s00425-007-0598-8

Castellarin, S. D., Pfeiffer, A., Sivilotti, P., Degan, M., Peterlunger, E., and Di Gaspero, G. (2007b). Transcriptional regulation of anthocyanin biosynthesis in ripening fruits of grapevine under seasonal water deficit. *Plant Cell Environ.* 30, 1381–1399. doi:10.1111/j.1365-3040.2007.01716.x

Choné, X., van Leeuwen, C., Dubourdieu, D., Gaudillère, J. P., and Gaudillere, J. P. (2001). Stem water potential is a sensitive indicator of grapevine water status. *Ann. Bot.* 87, 477–483. doi:10.1006/anbo.2000.1361

Costantini, E. A. C., Agnelli, A., Bucelli, P., Ciambotti, A., Dell'Oro, V., Natarelli, L., et al. (2013). Unexpected relationships between δ ^{13}C and wine grape performance in organic farming. *J. Int. Sci. Vigne Vin* 47, 269–285. doi:10.20870/oeno-one.2013.47.4.1556

Council of the European Union (2006). *Council Regulation (EC) No 510/2006 of 20 March 2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs*. Brussels, Belgium: Council of the European Union.

Dal Santo, S., Zenoni, S., Sandri, M., De Lorenzis, G., Magris, G., De Paoli, E., et al. (2018). Grapevine field experiments reveal the contribution of genotype, the influence of environment and the effect of their interaction (G×E) on the berry transcriptome. *Plant J.* 93, 1143–1159. doi:10.1111/tpj.13834

Deloire, A., Vaudour, E., Carey, V., Bonnardot, V., and Van Leeuwen, C. (2005). Grapevine responses to terroir: a global approach. *J. Int. Sci. Vigne Vin* 39, 149–162. doi:10.20870/oeno-one.2005.39.4.888

des Gachons, C. P., van Leeuwen, C., Tominaga, T., Soyer, J. P., Gaudillère, J. P., and Dubourdieu, D. (2005). Influence of water and nitrogen deficit on fruit ripening and aroma potential of Vitis vinifera L cv Sauvignon blanc in field conditions. *J. Sci. Food Agric.* 85, 73–85. doi:10.1002/jsfa.1919

Matthews, M. A. (2016). *Terroir and other myths of winegrowing*. Berkeley, CA: University of California Press.

OIV (2010). *General assembly of the international organisation of vine and wine. Resolution OIV/VITI 333/2010*. Paris, France: OIV.

Priori, S., Pellegrini, S., Perria, R., Puccioni, S., Storchi, P., Valboa, G., et al. (2019). Scale effect of terroir under three contrasting vintages in the Chianti Classico area (Tuscany, Italy). *Geoderma* 334, 99–112. doi:10.1016/j.geoderma.2018.07.048

Seguin, G. (1986). "Terroirs" and pedology of wine growing. *Experientia* 42, 861–873. doi:10.1007/bf01941763

Tardaguila, J., Baluja, J., Arpon, L., Balda, P., and Oliveira, M. (2011). Variations of soil properties affect the vegetative growth and yield components of "Tempranillo" grapevines. *Precis. Agric.* 12, 762–773. doi:10.1007/s11119-011-9219-4

Tramontini, S., van Leeuwen, C., Domec, J. C., Destrac-Irvine, A., Basteau, C., Vitali, M., et al. (2013). Impact of soil texture and water availability on the hydraulic control of plant and grape-berry development. *Plant Soil* 368, 215–230. doi:10.1007/s11104-012-1507-x

van Leeuwen, C., Friant, P., Choné, X., Tregoat, O., Koundouras, S., and Dubourdieu, D. (2004). Influence of climate, soil, and cultivar on terroir. *Am. J. Enol. Vitic.* 55, 207–217.

van Leeuwen, C., Schultz, H. R., Garcia de Cortazar-Atauri, I., Duchene, E., Ollat, N., Pieri, P., et al. (2013). Why climate change will not dramatically decrease viticultural suitability in main wine-producing areas by 2050. *Proc. Natl. Acad. Sci. U. S. A.* 110, E3051–E3052. doi:10.1073/pnas.1307927110

van Leeuwen, C., and Seguin, G. (2006). The concept of terroir in viticulture. *J. Wine Res.* 17, 1–10. doi:10.1080/09571260600633135

Vaudour, E. (2002). The quality of grapes and wine in relation to geography: notions of terroir at various scales. *J. Wine Res.* 13, 117–141. doi:10.1080/0957126022000017981

Vaudour, E., Costantini, E., Jones, G. V., and Mocali, S. (2015). An overview of the recent approaches to terroir functional modelling, footprinting and zoning. *SOIL* 1, 287–312. doi:10.5194/soil-1-287-2015

White, R. E. (2020). The value of soil knowledge in understanding wine terroir. *Front. Environ. Sci.* 8. doi:10.3389/fenvs.2020.00012

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Exploring Grapevine Phenology and High Temperatures Response Under Controlled Conditions

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Climate change has challenged growers and researchers alike to better understand how warm temperatures may impact winegrape plant development across varieties. Yet multi-variety studies present challenges. Here we review studies of controlled warming on winegrape varieties alongside a new study of the budburst and flowering phenology of 50 varieties of *Vitis vinifera* subsp. *vinifera* in the lab, with a small set of plants exposed to higher temperatures (20, 26, 30, 34, and 37°C mean temperatures in growth chambers) during flowering. We found few studies have examined more than one variety, which may be due to the challenge of growing diverse varieties together. Indeed, we found high variability in flowering success across varieties in the lab (28 out of 50 varieties had no flowering), which made it impossible to study variety-specific response to temperature. Across varieties, however, we found results in line with a literature review (which we also present): higher temperatures did not have a significant effect on the rate at which vines progressed through the flowering stage, but higher temperatures did correlate with flower abortion. These results suggest a potential decrease in winegrape yields in a warmer climate due to flower abortion, but also highlight the challenges of understanding heat responses across many varieties.

Keywords: phenology, climate change, heat stress, flowering, lab conditions, *Vitis vinifera* subsp. *vinifera*

INTRODUCTION

As the climate changes, the viticulture industry needs to adapt to shifting terroir. Terroir – the critical link between the flavor and style of a wine and the characteristics of the environment in which it is grown – is shaped strongly by climate, and the matching of climates to varieties (Van Leeuwen et al., 2019). Thus, as climate change continues to raise temperatures in winegrowing regions across the world, the viticulture industry will be continually challenged to adapt to new terroirs over future decades. Already, the industry has shifted growing areas toward the poles and higher elevations to maintain ideal growing temperatures for winegrapes (Mozell and Thach, 2014; Wang et al., 2020). This trend is predicted to continue (Schultz and Jones, 2010;

Hannah et al., 2013), raising concerns that vineyards could move to land that is currently conserved for biodiversity and ecosystem services (Hannah et al., 2013).

Alternatively, vineyards could take advantage of the high geno- and phenotypic diversity that already exists by planting varieties better suited to the new climate (Ollat et al., 2015, 2016; Wolkovich et al., 2017; Morales-Castilla et al., 2020) or breeding new varieties (Myles, 2013; Duchêne, 2016). *Vitis vinifera* subsp. *vinifera* (winegrape) has at least 6000 genetically distinct varieties grown for many purposes, but only ∼1100 are grown currently by the viticulture industry, and an even smaller number dominate the global market (Lacombe, 2012; Anderson, 2013). However, for this adaptation to be effective, growers need better information on how different varieties fare in warmer climate regimes, with phenology being one important component (Ollat et al., 2016).

Studying the phenology of different varieties of winegrapes would help viticulturists better adapt to climate change, because winegrape phenology is extremely sensitive to temperature (Parker et al., 2011, 2013; Jones, 2013; García de Cortázar-Atauri et al., 2017). Timing for leafout and flowering of diverse plant species has advanced six to 20 days in the last 30–40 years of warming (Root et al., 2003; Menzel et al., 2006), equivalent to 4–6 days per °C. A similar advance is seen for winegrape harvest dates, which can change about 6 days per °C (Cook and Wolkovich, 2016; Labbé, 2019). The time between flowering and veraison also decreased by a little more than 1 day per °C (Duchêne and Schneider, 2005). In winegrapes, phenological timing varies across varieties, and this variability could be used to better adapt to future climates. Generally, timing of phenology can vary from 3 to 6 weeks across varieties (Boursiquot et al., 1995; Wolkovich et al., 2017).

However, most varieties still have little phenological data and far fewer varieties have data from many different environments. In this context, it is difficult to describe where many varieties could best be grown and how they respond to higher temperatures during critical phenological phases, such as flowering. While recent efforts have greatly expanded our resources for understanding phenological responses to climate in the field across varieties – yielding information on approximately 100 varieties (Parker et al., 2011, 2013) this is still less than 10% of currently planted varieties. For growers to select varieties for adapting to shifting terroirs, they will need information on more varieties and across diverse temperature regimes.

A first step toward this goal is research on an increased number of varieties and an understanding of whether phenology in semi-artificial conditions (i.e., greenhouses, labs, and growth chambers), where temperatures can be controlled more easily, matches field-based phenology. To date, much research has focused on a limited number of varieties (Sepúlveda et al., 1986; Mullins, 1992), making it difficult to know how much results for one variety can be extrapolated to another. Yet, if a greater diversity of varieties can be grown in lab conditions, lab studies could quickly increase our understanding across varieties. Further, if lab phenology appears similar to field phenology, it would suggest such results could be relevant to field conditions. Beyond this first step then, researchers will want to examine how varying temperature regimes affect particular phenological stages.

Understanding how climate change will affect winegrape flowering may be a particularly important aspect of the overall effect on phenology and the impact of temperature on the flowering process will ultimately influence harvest yields. Studies of vegetative growth and photosynthesis in other perennial crops exposed to a range of temperatures show that extreme temperatures tend to slow or inhibit certain processes in the plants (Zaka et al., 2016, 2017), with temperatures in between extremes generally speeding development. In this context, we would expect that grapevine flowering development may similarly slow down at higher temperatures.

Here we address these issues through first, a literature review of warming studies on winegrape phenology to examine how many varieties have been studied, over which temperatures, and their findings, and second, our efforts to examine experimentally how temperature affects flowering in a variety-rich study. To explore this second issue, we had two major aims: (1) to test whether the phenological stages of budburst and leafout in lab conditions correlated with field phenology for 50 varieties in the lab, and (2) to examine the effect of higher temperatures on flowering development, by following the flowering response of a small subset of these varieties across mean temperatures of 20 to 37°C in growth chambers. Overall, we aim to provide both an overview of experiments to date, and to outline how our findings and challenges may guide future efforts to conduct variety-rich lab experiments.

MATERIALS AND METHODS

Literature Review

We conducted a literature review by searching Google Scholar, ISI Web of Science, and ScholarOneSearch for several searches, each search included "*Vitis vinifera*" combined with (AND) "heat tolerance*" OR "growth chamber" OR "phenolog*" OR "temperature manipulation." Then we reviewed papers that experimentally manipulated temperatures of growing grapevines and reported phenological responses (excluding all studies without experimental warming or of warming applied to dormant cuttings or plants or focused only on berry ripening). We additionally included any relevant papers of which we were aware that we did not find in these searches. While some studies included additional treatments (e.g., drought, CO_2 manipulation) we focus on results relating to warming and phenology.

Variety-Rich Study

Observations of field-grown winegrapes in the UC Davis Robert Mondavi Institute (RMI) Vineyard (Davis, CA, United States) using the modified Eichorn-Lorenz (EL) scale (Coombe, 1995) began 6 March 2015 and continued generally every 3–4 days until 2 April 2015, when almost all plants had reached EL stage 11 or higher (data and full methods available at: https://knb.ecoinformatics.org/view/ doi: 10.5063/F18G8J29). Dormant winegrape cuttings were then taken in December of 2015.

TABLE 1 | Literature review of studies applying experimental warming to winegrapes during development and following phenological responses.

Paper	Varieties	Type	Temperature	Effects	Vine age
Edwards et al., 2017	Shiraz	Field experimental warming (passive chambers)	2°C warming from average temperature (passive heating)	All aspects of vine phenology advanced	"mature"
Gouot et al., 2019	Shiraz	System to heat only aboveground parts of plants	+6°C at end of fruit set and again prior to veraison (immediate)	Photosynthesis decreased when heating led to 45°C temperatures, but not when only 40°C	7 years
Greer and Weedon, 2013	Semillon	Field experimental cooling	Some vines protected from 40+°C ambient temperatures (passive heating)	Heat delayed ripening	6 years
Greer and Weedon, 2014	Merlot, Chardonnay, Semillon	Growth chambers	20–40°C range, four treatments, post-veraison (temperatures raised in chamber 10 days after plants were allocated to chambers)	Varied by variety: Merlot: no effect on berry, Chardonnay: rapid expansion at 20 and 25°C but decline in size at 40°C, Semillon: expansion at 20 and 25°C but not at higher temps	5 years
Greer and Weston, 2010	Semillon	Growth chambers	40/25°C at flowering, fruit set, veraison and mid-ripening stages (immediate)	Heat did not affect leaf growth or stem extension, but flowers completely abscised. Berries treated at fruit set developed normally and those treated at veraison and mid-ripening stopped expanding and sugar content stopped increasing	3 years
Kadir, 2006	Semillon, Pinot Noir, Chardonnay, Cabernet-Sauvignon, Cynthiana (*Vitis aestivalis*)	Growth chambers	20–40°C range, three treatments (immediate)	Increase in vegetative growth for *V. vinifera* from 20 to 30°C, but most growth stunted at 40°C –*V. vinifera* affected less by high temperatures	1 year
Kliewar, 1977	Cabernet-Sauvignon, Tokay, Pinot Noir, Carignane	Growth chambers	35, 40°C warming during 2–8 days before to 12–18 days after bloom 25/20°C controls (immediate)	Variable effects on berry set and weight, depending on variety; no effect on rate of shoot growth	3–4 years
Petrie and Clingeleffer, 2005	Chardonnay	Field experimental warming (passive chambers)	Range of 3°C across treatments (passive heating)	Resulted in decrease in flowering of 15 to 25% due to temperature	"established vineyard"
Salazar-Parra et al., 2010	Tempranillo	Greenhouse	28/18°C vs. 24/14°C, day/night at veraison (immediate)	Warming shortened the time between grape veraison and full maturity	<1 year
Soar et al., 2009	Shiraz	Field experimental warming (chambers with fans)	6.5–7.3°C above ambient for 3 days (passive heating)	No effect on berry growth or sugar accumulation	10 years

We provide the rate at which temperature treatments were applied parenthetically.

Following collection, cuttings were chilled for 21 days (4°C) at the Arnold Arboretum (Boston, MA, United States), then forced in greenhouses in 26 cm diameter (9.6L) pots in January 2016. After several months of growth, on 27 May they were placed in growth chambers with day/night temperatures of 6/4°C and an 8-h photoperiod to induce dormancy, though the plants did not appear visibly dormant until 20 June 2016.

On 15 August 2016, the 351 potted cuttings were moved out of the chambers and into a greenhouse where the initial day temperature was 18.5 ± 1.5°C and night temperature was 16.75 ± 1.25°C. After the first week, the temperatures were slowly raised to 25.5 ± 2.5°C during the day and lowered to 10°C at night. The cuttings were pruned the day they were removed from the chambers so that each cutting had two spurs and each spur had two nodes. Then, the diameter of each spur and node and the distance between the two nodes on each spur were measured with calipers. About every 2 days, the plants' soil was checked for moisture, and they were watered as needed to keep soils moist. Starting 1 October, plants were also fertilized once a week with a 50% dilution.

Twice a week, beginning 22 August, each plant's development was recorded using the modified EL scale (Coombe, 1995) and soil moisture was measured with a probe in three locations in each pot. Each spur was kept at two shoots, but only the dominant shoot on each spur had observations recorded. Each shoot was trained up a stake for support. When an inflorescence had developed (EL stage 12), the plant was randomly assigned to one of five growth chambers if it was a part of the heat tolerance experiment (varieties were chosen for inclusion in the experiment to include a diversity of phenology from those varieties for which there were five or more replicates growing). Otherwise, observations on each plant continued in the greenhouse.

The five chambers all had a 12-h photoperiod with 800 $m^{-2}s^{-1}$ of fluorescent light, but varied in their temperature: Chamber 1 was set at 17/23°C Chamber 2 was set at 23/29°C, Chamber 3 was set at 27/33°C, Chamber 4 was set at 31/37°C, and Chamber 5 was set at 34/40°C (all temperatures given as night/day). Initially, CO_2 levels were set at 400 ppm during the day and 600 ppm at night, because plants respire at night, increasing CO_2 levels (we used 600 ppm given a review of the literature in natural and crop systems where we found little evidence of levels above 550 ppm near plants, e.g., Buchmann and Ehleringer (1998) and Mortazavi and Chanton (2002), though we did not find grape-specific studies). Each inflorescence was contained in a paper bag to collect the flower caps as they fell. Every 10 days, the plants and their assigned temperatures were rotated to a new chamber to minimize individual chamber effects on the experiment.

Observations of the percent of flower buds that flowered on each inflorescence (% flowering), leaf number, stem length, and number of fallen flower caps were made three times a week, along with soil moisture. On 19 September, it was noted that some inflorescence bags also contained aborted buds that had yet to flower, and thereafter observations of aborted buds were also recorded. Once a plant had reached 100% flowering, or, in the case of plants where the entire inflorescence had abscised, each plant had spent a minimum 14 days in the chamber, it was returned to the greenhouse. No further observations were made once no more plants were developing inflorescences and all plants in the chambers had finished flowering (data available at: https://knb.ecoinformatics.org/view/doi:10.5063/F1TM78HS).

To determine if there was any correlation between the chamber temperatures and the other variables, we used ANOVA. Linear regression was used to compare the development of the plants in the greenhouse with the data collected in the RMI Vineyard growing season. All analyses were performed in R version 3.3.3 (R Core team, 2013). Given limited replicates per variety all analyses of the growth chamber study were done across varieties.

RESULTS

Literature Review

Most studies (7/10) examined only one variety, while at most one study examined five varieties. Certain varieties were studied often (e.g., Semillon, Shiraz, and Cabernet-Sauvignon); given the overlap in varieties across studies, all 10 studies yielded information on only a total of 10 varieties (**Table 1**). Experimental warming was split between being applied in the vineyard (through passive and active warming) or in the lab (growth chambers or greenhouses) with temperatures generally ranging from 20 to 40°C, while some field conditions exceeded 40°C. Warming generally advanced phenology, save for one field study that showed temperatures above 40°C delayed veraison (Greer and Weedon, 2013). Studies focused on flowering found decreased flowering at higher temperatures applied near budburst (Petrie and Clingeleffer, 2005) and flower abscission at higher temperature applied during flowering (Greer and Weston, 2010).

Variety-Rich Study

The plants underwent budbreak (EL 4) between 17 August and 6 September (mean = 29 August) and leafout (EL 7) between 22 August and 22 September (mean = 4 September). Budbreak and leafout timing among the varieties were similar in the lab and field (**Figure 1**, budburst: $F(1,47) = 14.55$, $p < 0.001$; leafout: $F(1,47) = 18.51$, $p < 0.001$). The first inflorescence formed on 5 September, and 51 plants reached this stage (EL 12) later, with substantial variation in terms of the number of plants of each variety that flowered at all. Most varieties (28/50 total) did not form inflorescences, while for a few varieties nearly half of the plants underwent flowering (e.g., Sauvignon Blanc, Tempranillo, Verdelho). Due to this high variation in inflorescence appearance, only 26 of the flowering plants were used in the experiment corresponding to 10 varieties.

Given the low number of plants that formed inflorescence, most varieties could be placed in only one or two temperature treatments (with very low or no replication per variety: chamber 1 (mean of 20°C) had one plant each of Cabernet-Sauvignon, Durif, Sauvignon Blanc, and Verdelho. Chamber 2 (mean of 26°C) had one plant each of Durif, Pinot Gris, Sauvignon Blanc, and Verdelho. Chamber 3 (mean of 30°C) had three Durif plants, then one plant each of Gewürztraminer, Tempranillo,

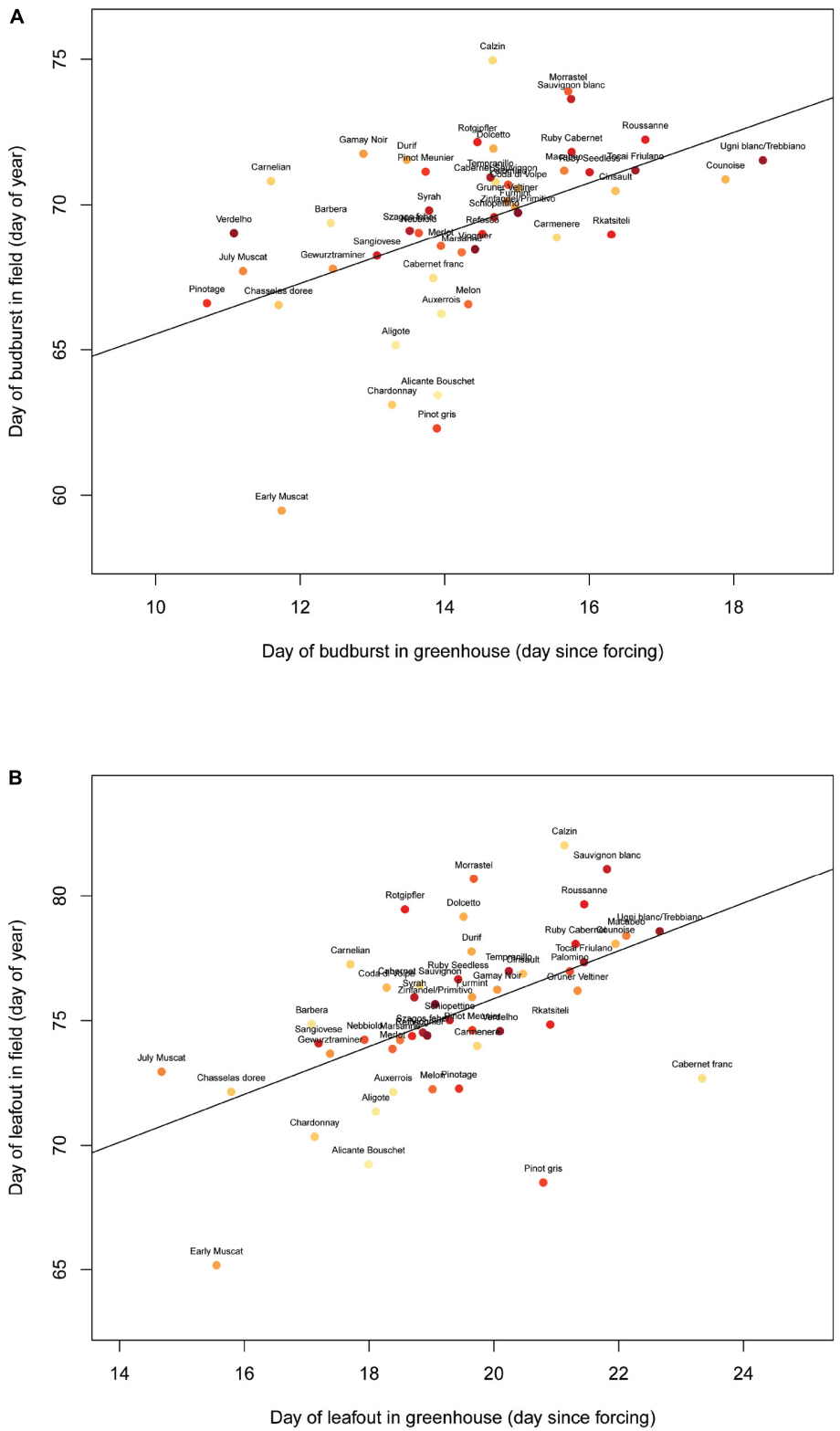

FIGURE 1 | Day of budburst **(A)** and leafout **(B)** in the Robert Mondavi Institute Vineyard (Davis, CA, United States) from the 2015 growing season correlates to the day of budburst ($F(1,47) = 14.55$, $p < 0.001$) and leafout ($F(1,47) = 18.51$, $p < 0.001$) in greenhouse conditions across 50 varieties (each point represents a different variety that was grown both in the vineyard and in the greenhouse).

and Verdelho. Chamber 4 (mean of 34°C) had two Tempranillo plants, then one each of Dolcetto, Pinot Gris, Sauvignon Blanc, Syrah, and Verdelho. Chamber 5 (mean of 37°C) had two Tempranillo plants, and one each of Sauvignon Blanc, Verdelho, and Vinhão). Because of the limited number of replicated per variety, we do not report variety-specific estimates and all statistics are done across varieties. Plants that had thicker spurs were more likely to develop inflorescence ($Z(340) = 2.21$, $p = 0.03$), and more likely to reach 50% flowering (**Figure 2**, $Z(340) = 2.85$, $p = 0.004$).

Soil moisture in the chambers varied by chamber temperature ($F(1,24) = 8.05$, $p = 0.01$), ranging from 69 to 76% over time. There was no directional relationship between the moisture levels and the chamber temperature (i.e., the warmest chambers were not the driest) and means were similar across treatments, ranging from 71 to 74%.

There was also no directional relationship between chamber temperature and either change in stem length or leaf appearance rate (stem length: $F(1,24) = 0.53$, $p = 0.47$; leaf appearance: $F(1,24) = 0.05$, $p = 0.83$).

Chamber temperatures did not affect the time it took for the plants to reach 10% and 50% flowering and there was no trend in the duration of flowering (**Figure 3**, 10%: $F(1,20) = 0.43$, $p = 0.52$; 50%: $F(1,15) = 0.50$, $p = 0.49$). Within treatments, the number of days after forcing it took plants to reach 10% flowering ranged from 34 to 51 days (mean = 42.6 ± 0.9).

The number of flower buds aborted per plant was significantly affected by the chamber temperature (**Figure 3**, $F(1,24) = 7.43$, $p = 0.01$). The two warmest chambers saw the greatest number of flower buds lost during the time in the chamber, with the greatest average number of flower buds aborted seen in 37°C treatment (mean number of flower buds aborted at 20°C: 4.5, 26°C: 2.8, 30°C: 5.8, 34°C: 27.6, 37°C: 57.3).

DISCUSSION

Increasingly, winegrape diversity is suggested as a way for growers to cope with warming, but we know little about how experimental warming temperature differentially affects most varieties (Ollat et al., 2015, 2016; Wolkovich et al., 2017). Research to date has focused on very few (only 10 according to our literature review) varieties, but suggests responses vary depending on variety. For example, Greer and Weedon (2014) found a curvilinear ripening response to temperature (with warmer temperatures speeding development up to some high temperature, above which development slowed) across three varieties – but the temperature yielding the highest ripening

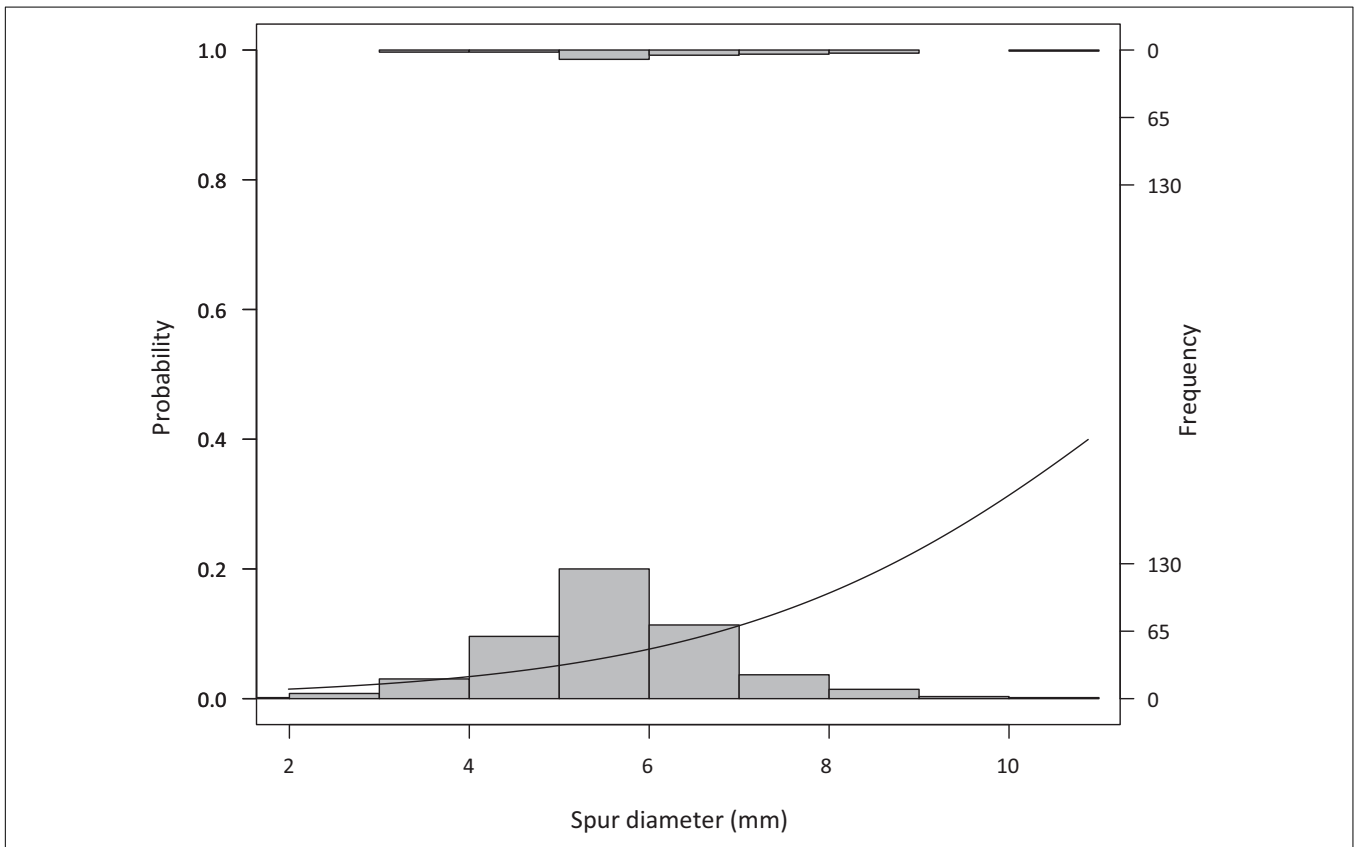

FIGURE 2 | Spur diameter in greenhouse-grown vines (measured when plants were removed from dormancy) related to the probability that a plant would reach 50% flowering ($Z(340) = 2.85$, $p = 0.004$), with larger spur vines more often reaching 50% flowering. Histograms show the vines that did not reach 50% flowering (recorded in this analysis as 0 values, bottom) and those that did reach 50% flowering (recorded in this analysis as 1 values, top).

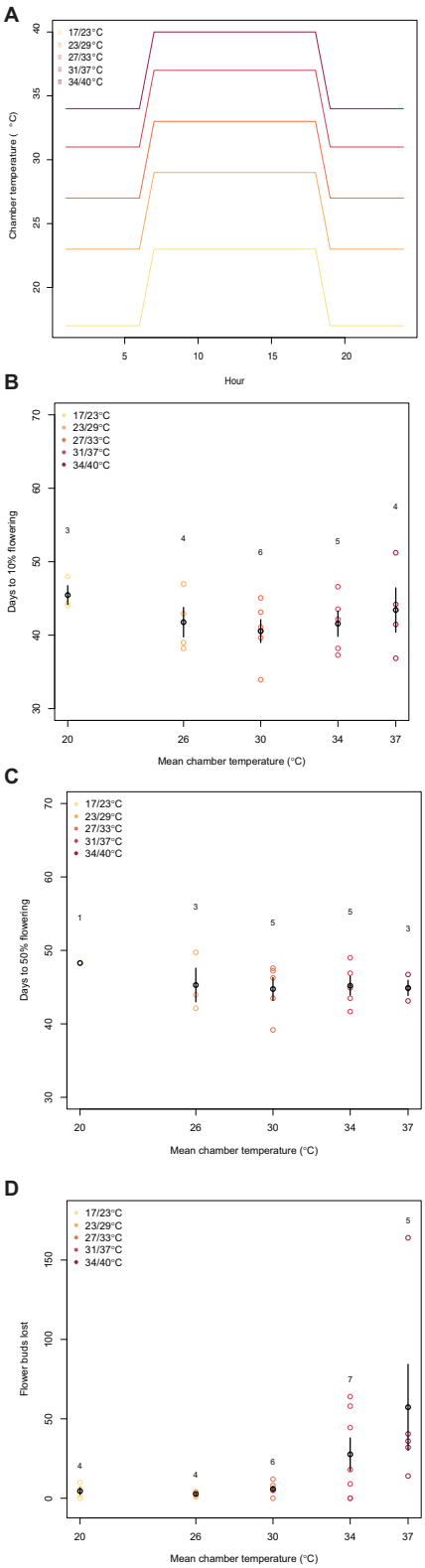

FIGURE 3 | These figures illustrate the relationship between mean chamber temperature (treatments shown in panel **(A)** and **(B)** the days it took the plants *(Continued)*

FIGURE 3 | Continued
to reach 10% flowering ($F(1,20) = 0.43$, $p = 0.52$), **(C)** the days it took the plants to reach 50% flowering (50%: $F(1,15) = 0.50$, $p = 0.49$), or **(D)** the number of flower buds lost while in the chamber ($F(1,24) = 7.43$, $p = 0.01$). The black points and bars show the average and error in each chamber. The number above each chamber's data is the sample size. The colored points represent individual plants. The legend in the top left corner gives the night/day temperature for each chamber.

varied for each variety (25, 35, and 40°C for Chardonnay, Semillion, and Merlot, respectively). Such variation is critical for growers who want to adapt to warming by shifting varieties, but to make useful variety recommendations we need more information on how temperature affects development across varieties and developmental stages. Our lab work on 50 varieties, however, highlights the challenges of growing diverse varieties for experimental research.

Effects of High Temperatures on Winegrape Flowering

Our lab work to examine how temperature affects flowering across diverse varieties failed to produce enough grapevines to study variety-specific effects. Yet, in pooling results across varieties, we found trends in line with previous studies.

Overall, we studied the effects of temperatures between a minimum of 17°C and maximum of 40°C (means of 20 to 37°C) on flowering for 26 winegrape plants. We found no directional relationship between temperature and soil moisture, stem length, leaf number, or the number of days it took to reach 10 or 50% flowering. Contrary to expectations of most phenological models (García de Cortázar-Atauri et al., 2010; Cuccia et al., 2014) and one previous growth chamber studies (Buttrose and Hale, 1973), we found that flowering phenology was not significantly delayed in either the coldest or warmest chambers. We expected development would slow (and thus phenology delay) at temperature extremes, especially at our upper temperature extreme of 37°C, however, phenology should generally advance until that extreme temperature. Our results suggest 37°C is not high enough to induce delays, a result in line with much of our literature review which found growth and phenology generally advanced up to 40°C (**Table 1**). Further, our results support previous work, which found that plants in the hotter treatments aborted a higher number of flowers than those in the cooler treatments (Greer and Weston, 2010). This abortion, because it translated to fewer observations of higher percentages of flowering (i.e., 50%), may have limited detection of slowed phenology at higher temperatures. Furthermore, our plants were only exposed to the higher temperatures during flowering, not before, which could have diminished potential differences in timing of phenology during that developmental phase.

The majority of literature on winegrape heat tolerance focuses on the effects of heat on berry ripening. In their 2010 study of Semillon winegrapes, Greer and Weston noted that plants treated with elevated temperatures at fruit set were much less vulnerable and suffered few ill-effects when compared with those treated at flowering, veraison, and

mid-ripening. When heat-treated at fruit set, berry growth was unimpeded and sugar content increased normally. This could mean that winegrapes are more vulnerable to high temperatures during certain periods of development, i.e., flowering. If winegrapes are especially susceptible to heat during flowering, viticulturists will have to take extra precautions during this period to ensure the survival of the flowers through to fruit set.

Although we did not measure fruit-set, future studies may want to investigate how it could be affected by elevated temperatures during the flowering period. There could be a delay in response between the period of warming and the effects of high temperatures that was not seen in our experiment because the plants were heated during the developmental phase in which we were interested. Continuing observations through fruit-set could be an important next step to help understand more exactly how harvest yields will be impacted in a warming climate.

Utility of Lab-Grown Winegrape Plants for Future Research

Because the majority of the plants' development did not form inflorescences (EL stage 12), sample sizes for our heat experiment were smaller than planned (each chamber had four to six plants). This meant there were not enough plants of each variety in each chamber to test for a difference in varietal response to the heat treatments, and instead we analyzed our findings across varieties (as most varieties were only represented in a single treatment). Still, it is important to note that we studied ten different varieties in the chambers, which greatly increased the genetic diversity of the experiment. It has been shown that controlled ecological experiments in labs that include greater genetic diversity are more easily replicated (Milcu et al., 2018).

Further, we found high variation in flowering success – plants with larger spurs were more likely to form inflorescence and flower and some varieties were far more successful in flowering than others. This suggests plants with greater carbohydrate reserves were more likely to develop inflorescence and flower, similar to the results of Eltom (2013) on the effects of girdling and leaf removal on inflorescence development, but with additional variation across varieties, as other studies have found (Lebon et al., 2005). Thus, future experiments may want to (at least initially) focus lab efforts on these more successful varieties and tease out high and low temperature limits to help guide further research.

The rate of development seen in the plants grown in the greenhouse was significantly correlated with that seen in the winegrapes grown in the Robert Mondavi Institute Vineyard, from which the cuttings in this experiment were taken (**Figure 1**). This suggests that the overall progression and timing of phenological development was not dramatically altered by the lab setting and supports the use of potted plants in the lab used alongside field data to better understand and predict winegrape responses to climate change. Our finding that plants with larger spurs were more likely to flower, however, suggests that our results regarding flower development in the greenhouse and flowering (and flower abortion) in the growth chambers should be interpreted cautiously.

Our vines, taken from field cuttings, were in only their first growing season, and this represents a major limitation of our study. We expect flowering success across varieties would be greater for older, larger vines, and our findings should be interpreted cautiously until further studies are completed on older vines. In the literature, studies vary in using <1 year-old potted cutting, to 3-to 5-year-old potted vines, to established vineyard plants. This diversity of vine age across studies that also vary treatments makes it difficult to attribute variation in findings to age, but our results suggest older vines may be most relevant and useful for studies on heat tolerance and warming effects.

While this study was unable to adequately address varietal differences in response to warming as a result of climate change, it provided valuable insight into challenges of variety-rich winegrape studies. Based on the outcome of our study, we recommend the following strategies to improve the success of similar future studies: (1) Use older vines or those with thicker diameters (which indirectly corresponds to greater carbohydrate reserves) to ensure a higher number of plants form inflorescences and undergo flowering. (2) Consider mesh bags to trap flowers; because we contained inflorescences in paper bags, we may have restricted air flow during a critical period of development, limiting photosynthesis. (3) Examine effects of gradual versus sudden temperature increases. Providing a transitional period for plants when they are moved into chambers and raising temperatures gradually could prevent shock or stress on the plants that could exacerbate flower abscission, but a more sudden temperature changes may be relevant for weather changes with climate change (Gouot et al., 2019).

CONCLUSION

Helping growers adapt to shifting terroirs requires research on a greater diversity of *Vitis vinifera* varieties across diverse temperature regimes. Here we showed that budburst and leafout phenology of 50 varieties grown in the field correlated with field-based phenology and that higher temperatures can negatively impact flowering. While heat treatments during flowering did not affect the phenology of the grapes we studied, we found a significant impact from the elevated temperatures on flower abortions, in line with previous studies, which could lead to substantial negative impacts on yield. Despite the difficulties we faced implementing a variety-rich experiment, lessons we learned can inform future studies to increase success and provide further guidance for academics and professionals alike. Our findings underscore the importance of modeling more than the plants' phenology to fully understand the impacts climate change will have on the viticulture industry. As data across more diverse varieties and temperature regimes increases,

it can help support mapping when and where different varieties may perform best as warming continues.

DATA AVAILABILITY STATEMENT

Greenhouse and growth chamber datasets available at https://knb.ecoinformatics.org/view/urn%3Auuid%3A59f80d14-bc09-49a6-8143-0e2823bab9a2. Field datasets from UC Davis available at https://knb.ecoinformatics.org/view/doi:10.5063/F18G8J29.

AUTHOR CONTRIBUTIONS

EW and NM performed the analysis and designed the methods for the experiment with input from IGCA, AP, and MW. NM collected the data and wrote the manuscript. EW, IGCA, AP, and MW edited the manuscript. All authors contributed to the article and approved the submitted version.

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REFERENCES

Anderson, K. (2013). *Which Winegrape Varieties are Grown Where?: a Global Picture*. Adelaide: University of Adelaide Press.

Boursiquot, J. M., Dessup, M., and Rennes, C. (1995). Distribution des principaux caracteres phenologiques, agronomiques et technologiques chez *Vitis vinifera* L. *Vitis* 34, 31–35.

Buchmann, N., and Ehleringer, J. R. (1998). CO_2 concentration profiles, and carbon and oxygen isotopes in C3 and C4 crop canopies. *Agric. For. Meteorol.* 89, 45–58. doi: 10.1016/S0168-1923(97)00059-2

Buttrose, M. S., and Hale, C. R. (1973). Effect of temperature on development of the grapevine inflorescence after bud burst. *Am. J. Enol. Vitic.* 24:14.

Cook, B. I., and Wolkovich, E. M. (2016). Climate change decouples drought from early wine grape harvests in France. *Nat. Clim. Change* 6, 715–719. doi: 10.1038/nclimate2960

Coombe, B. G. (1995). Growth stages of the grapevine: adoption of a system for identifying grapevine growth stages. *Aust. J. Grape Wine Res.* 1, 104–110. doi: 10.1111/j.1755-0238.1995.tb00086.x

Cuccia, C., Bois, B., Richard, Y., Parker, A. K., García de Cortázar-Atauri, I., Van Leeuwen, C., et al. (2014). Phenological model performance to warmer conditions: application to Pinot Noir in Burgundy. *J. Int. Sci. Vigne Vin.* 48, 169–178. doi: 10.20870/oeno-one.2014.48.3.1572

Duchêne, E. (2016). How can grapevine genetics contribute to the adaptation to climate change? *OENO One* 50:98. doi: 10.20870/oeno-one.2016.50.3.98

Duchêne, E., and Schneider, C. (2005). Grapevine and climatic changes: a glance at the situation in Alsace. *Agron. Sustain. Dev.* 25, 93–99. doi: 10.1051/agro:2004057

Edwards, E. J., Unwin, D., Kilmister, R., and Treeby, M. (2017). Multi-seasonal effects of warming and elevated CO_2 on the physiology, growth and production of mature, field grown, *Shiraz grapevines*. *OENO One* 51:127. doi: 10.20870/oeno-one.2016.0.0.1586

Eltom, M. (2013). *Influence of Temperature and Carbohydrate Availability on the Bunch Architecture of Vitis vinifera L. Sauvignon Blanc*. Ph. D thesis, Lincoln University, Lincoln.

García de Cortázar-Atauri, I., Chuine, I., Donatelli, M., Parker, A., and van Leeuwen, C. (2010). "A curvilinear phenological model to study impacts of climate change on grapevine," in *Proceedings of "Agro2010 the XIth ESA Congress"*, Montpellier, France, 907–908.

García de Cortázar-Atauri, I., Duchêne, E., Destrac-Irvine, A., Barbeau, G., Rességuier, L., de Lacombe, T., et al. (2017). Grapevine phenology in France: from past observations to future evolutions in the context of climate change. *OENO One* 51:1622. doi: 10.20870/oeno-one.2017.51.2.1622

Gouot, J. C., Smith, J., Holzapfel, B., and Barril, C. (2019). Single and cumulative effects of whole-vine heat events on Shiraz berry composition. *OENO One* 53, 171–187. doi: 10.20870/oeno-one.2019.53.2.2392

Greer, D., and Weedon, M. (2014). Temperature-dependent responses of the berry developmental processes of three grapevine (*Vitis vinifera*) cultivars. *N. Z. J. Crop Hortic. Sci.* 42, 233–246. doi: 10.1080/01140671.2014.894921

Greer, D. H., and Weedon, M. M. (2013). The impact of high temperatures on *Vitis vinifera* cv. Semillon grapevine performance and berry ripening. *Front. Plant Sci.* 4:491. doi: 10.3389/fpls.2013.00491

Greer, D. H., and Weston, C. (2010). Heat stress affects flowering, berry growth, sugar accumulation and photosynthesis of *Vitis vinifera* cv. Semillon grapevines grown in a controlled environment. *Funct. Plant Biol.* 37, 206–214. doi: 10.1071/FP09209

Hannah, L., Roehrdanz, P. R., Ikegami, M., Shepard, A. V., Shaw, M. R., Tabor, G., et al. (2013). Climate change, wine, and conservation. *Proc. Natl. Acad. Sci. U.S.A.* 110, 6907–6912. doi: 10.1073/pnas.1210127110

Jones, G. V. (2013). "Winegrape phenology," in *Phenology: An Integrative Environmental Science*, ed. M. D. Schwartz (Berlin: Springer), 563–584. doi: 10.1007/978-94-007-6925-0_30

Kadir, S. (2006). Thermostability of photosynthesis of *Vitis aestivalis* and *V. vinifera*. *J. Am. Soc. Hortic. Sci.* 131, 476–483. doi: 10.21273/JASHS.131.4.476

Kliewar, W. M. (1977). Effect of high temperatures during the bloom-set period on fruit-set, ovule fertility, and berry growth of several grape cultivars. *Am. J. Enol. Vitic.* 28, 215–222.

Labbé, T. P. (2019). The longest homogenous series of grape harvest dates, Beaune 1354-2018, and its significance for the understanding of past and present climate. *Clim. Past* 65, 1485–1501. doi: 10.5194/cp-15-1485-2019

Lacombe, T. (2012). "Contribution à l'étude de l'histoire évolutive de la vigne cultivée (*Vitis vinifera* L.) par l'analyse de la diversité génétique neutre et de gènes d'intérêt," in *Thèse en Biodiversité et écologie*, Institut National d'Etudes Supérieures Agronomiques de Montpellier, 94.

Lebon, G., Duchêne, E., Brun, O., and Clément, C. (2005). Phenology of flowering and starch accumulation in Grape (*Vitis vinifera* L.) cuttings and vines. *Ann. Bot.* 95, 943–948. doi: 10.1093/aob/mci108

Menzel, A., Sparks, T. H., Estrella, N., Koch, E., Aasa, A., Ahas, R., et al. (2006). European phenological response to climate change matches the warming pattern. *Glob. Change Biol.* 12, 1969–1976. doi: 10.1111/j.1365-2486.2006.01193.x

Milcu, A., Puga-Freitas, R., Ellison, A. M., Blouin, M., Scheu, S., Freschet, G. T., et al. (2018). Genotypic variability enhances the reproducibility of an ecological study. *Nat. Ecol. Evol.* 2, 279–287. doi: 10.1038/s41559-017-0434-x

Morales-Castilla, I., García de Cortázar-Atauri, I., Cook, B. I., Lacombe, T., Parker, A., van Leeuwen, C., et al. (2020). Diversity buffers winegrowing regions from climate change losses. *Proc. Natl. Acad. Sci. U.S.A.* 117, 2864–2869. doi: 10.1073/pnas.1906731117

Mortazavi, B., and Chanton, J. P. (2002). Carbon isotopic discrimination and control of nighttime canopy δ 18 O-CO_2 in a pine forest in the southeastern United States: isotopic discrimination in a pine forest. *Glob. Biogeochem. Cycles* 16:8. doi: 10.1029/2000GB001390

Mozell, M. R., and Thach, L. (2014). The impact of climate change on the global wine industry: challenges & solutions. *Wine Econ. Policy* 3, 81–89. doi: 10.1016/j.wep.2014.08.001

Mullins, M. G. (1992). *Biology of the Grapevine*. Cambridge: Cambridge University Press.

Myles, S. (2013). Improving fruit and wine: what does genomics have to offer? *Trends Genet.* 29, 190–196. doi: 10.1016/j.tig.2013.01.006

Ollat, N., Quénol, H., Barbeau, G., van Leeuwen, C., Darriet, P., García de Cortázar-Atauri, I., et al. (2015). Adaptation to climate change: strategic issues to face for the French wine industry. *Web Confer.* 50:01020.

Ollat, N., Touzard, J.-M., and van Leeuwen, C. (2016). Climate change impacts and adaptations: new challenges for the wine industry. *J. Wine Econ.* 11, 139–149. doi: 10.1017/jwe.2016.3

Parker, A., García de Cortázar-Atauri, I., Chuine, I., Barbeau, G., Bois, B., Boursiquot, J.-M., et al. (2013). Classification of varieties for their timing of flowering and veraison using a modelling approach: a case study for the grapevine species *Vitis vinifera* L. *Agric. For. Meteorol.* 180, 249–264. doi: 10.1016/j.agrformet.2013.06.005

Parker, A. K., De Cortazar-Atauri, I. G., Van Leeuwen, C., and Chuine, I. (2011). General phenological model to characterise the timing of flowering and veraison of *Vitis vinifera* L. *Aust. J. Grape Wine Res.* 17, 206–216. doi: 10.1111/j.1755-0238.2011.00140.x

Petrie, P. R., and Clingeleffer, P. R. (2005). Effects of temperature and light (before and after budburst) on inflorescence morphology and flower number of Chardonnay grapevines (*Vitis vinifera* L.). *Aust. J. Grape Wine Res.* 11, 59–65. doi: 10.1111/j.1755-0238.2005.tb00279.x

R Core team (2013). *R: A Language and Environment for Statistical Computing*. Vienna: R Core team.

Root, T. L., Price, J. T., Hall, K. R., Schneider, S. H., Rosenzweig, C., and Pounds, J. A. (2003). Fingerprints of global warming on wild animals and plants. *Nature* 421:57. doi: 10.1038/nature01333

Salazar-Parra, C., Aguirreolea, J., Sánchez-Díaz, M., Irigoyen, J., and Morales, F. (2010). Effects of climate change scenarios on *Tempranillo grapevine* (*Vitis vinifera* L.) ripening: response to a combination of elevated CO_2 and temperature, and moderate drought. *Plant Soil* 337, 179–191. doi: 10.1007/s11104-010-0514-z

Schultz, H. R., and Jones, G. V. (2010). Climate induced historic and future changes in viticulture. *J. Wine Res.* 21, 137–145. doi: 10.1080/09571264.2010.530098

Sepúlveda, G., Kliewer, W. M., and Ryugo, K. (1986). Effect of high temperature on grapevines (*Vitis vinifera* L.). I. translocation of 14C-photosynthates. *Am. J. Enol. Vitic.* 37, 13–19.

Soar, C., Collins, M., and Sadras, V. (2009). Irrigated Shiraz vines (*Vitis vinifera*) upregulate gas exchange and maintain berry growth in response to short spells of high maximum temperature in the field. *Funct. Plant Biol.* 36, 801–814. doi: 10.1071/FP09101

Van Leeuwen, C., Destrac-Irvine, A., Dubernet, M., Duchêne, E., Gowdy, M., Marguerit, E., et al. (2019). An update on the impact of climate change in viticulture and potential adaptations. *Agronomy* 9:514. doi: 10.3390/agronomy9090514

Wang, J., Zhang, X., Su, L., Li, H., Zhang, L., and Wei, J. (2020). Global warming effects on climate zones for wine grape in Ningxia region, China. *Theor. Appl. Climatol.* 140, 1527–1536. doi: 10.1007/s00704-020-03170-y

Wolkovich, E. M., Burge, D. O., Walker, M. A., and Nicholas, K. A. (2017). Phenological diversity provides opportunities for climate change adaptation in winegrapes. *J. Ecol.* 105, 905–912. doi: 10.1111/1365-2745.12786

Zaka, S., Ahmed, L. Q., Escobar-Gutiérrez, A. J., Gastal, F., Julier, B., and Louarn, G. (2017). How variable are non-linear developmental responses to temperature in two perennial forage species? *Agric. For. Meteorol.* 232, 433–442. doi: 10.1016/j.agrformet.2016.10.004

Zaka, S., Frak, E., Julier, B., Gastal, F., and Louarn, G. (2016). Intraspecific variation in thermal acclimation of photosynthesis across a range of temperatures in a perennial crop. *AoB Plants* 8:plw035. doi: 10.1093/aobpla/plw035

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Counteracting the Negative Effects of Copper Limitations Through the Biostimulatory Action of a Tropical Plant Extract in Grapevine Under Pedo-Climatic Constraints

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In southern Mediterranean areas, vineyards are facing the combination of increasing air temperature, drought and frequency of extreme events (e.g., heat waves) due to climate change. Since most of the berry growth and ripening phases occur during the aridity period, such environmental constraints are responsible for limitations in yield and berry quality. Within this scenario, to achieve vineyard sustainability, renewed approaches in vineyard management have been proposed and the use of plant biostimulants seems a prominent and environmental friendly practice. The aim of this study was to test four combinations of a tropical plant extract and conventional chemicals for disease control on morpho-anatomical, physiological, biochemical and berry quality in *Vitis vinifera* L. subsp. *vinifera* "Aglianico." In particular, we aimed to evaluate the possibility to counteract the negative effects of the reductions in copper distribution, by applying the tropical plant extract enriched with: micronutrients, enzymes involved in the activation of natural defense, aminoacids, and vitamins. The halved dose of Cu in combination with the tropical plant extract allowed maintaining a reduced vegetative vigor. In the second year of treatment, the addition of the plant extract significantly improved leaf gas exchanges and photochemistry as well as the synthesis of photosynthetic pigments. At berry level, the plant extract induced an increase in phenolics accompanied by a decrease in soluble sugars. The overall results showed that the expected differences in growth performance and productivity in vines are linked to different eco-physiological and structural properties induced by the various treatments. The tropical plant extract also primed plant defenses at the leaf and fruit levels, mainly due to modifications of some structural and biochemical traits, respectively.

Keywords: plant-based biostimulant, copper, eco-physiology, functional anatomical traits, soil-plant-atmosphere continuum, *Vitis vinifera* L.

INTRODUCTION

The scientists and extension specialists are called to make local farming communities and crop production more resilient to climate change. There is an urgent need for developing mitigation and adapting solutions to cope with the scarcity of natural resources and to the increase yield stability under multiple stress conditions. Such objectives can be achieved through a sustainable agriculture targeted to improve the use efficiency of farm resources, simultaneously increasing crop yield and quality. Indeed, the climate conditions for world winegrowing regions are expected to change (Webb et al., 2013) with severe repercussions on viticulture which is an high-income agricultural sector (Jones and Webb, 2010; Goode, 2012).

In Europe, where viticulture is one of the most important agricultural sectors (average annual production of 168 million hectoliters, 54% of global consumption; FAOSTAT, 2020), a decrease in rainfall, associated with an increase in temperature, is expected especially in the Mediterranean region (IPCC, 2014). The combination of high air temperature and water deficit, coupled with marked inter-annual and intra-annual climate variability and scarce water resources (Costa et al., 2007; Rogiers and Clarke, 2013; Lopes et al., 2014; Valverde et al., 2015), leads to a severe depletion in soil water availability resulting in an important vulnerability of rainfed agriculture. Mediterranean viticulture is highly threatened by such projected environmental limitations because berry growth and ripening occur under conditions of high air temperature and soil water deficit which may limit yield and berry quality (Medrano et al., 2003; Chaves et al., 2007, 2010; Lereboullet et al., 2013, 2014). Moreover, this phenomenon is exacerbated by the competition for water resource with other sectors (e.g., industry) and by the prohibition of irrigation in most "Demarcation of controlled production areas" (DOC).

Other than climate change, European viticulture is facing also a legislative rearrangement which includes several restrictions in the use of chemical compounds suitable for pest and disease management. In particular, copper compounds, that have been used for more than one century mainly to control downy mildew in vineyards at relatively high rates depending on climatic conditions, caused a metallic copper accumulation in the topsoil of many vineyards (Rusjan et al., 2007), especially in organic cultivation. Therefore, the use of copper fungicides has been strongly restricted by the European Commission, firstly by the UE Reg. n. 889//2008, that allowed to use no more than 6 kg ha^{-1} of metallic copper per year, and recently even more by UE Reg. n. 1981/2018 that limits the amount of metallic copper to the maximum cumulated threshold of 28 kg ha^{-1} in seven consecutive years. However, despite its unfavorable ecotoxicological profile, copper is still tolerated due to its distinctiveness as wide spectrum fungicide, at least until an alternative product or control strategy will be identified (Dagostin et al., 2011). During the last two decades, significant efforts have been done by researchers and by European agricultural policy makers to comply with environmental safety and organic farming needs in the screening and evaluation of several alternatives to metallic copper compounds, including both inorganic substances at lower concentration of copper and plant extracts (Cohen et al., 2006; Chuang et al., 2007).

In this framework, securing yield stability and improving berry quality under multiple/combined stressful conditions (i.e., high temperature and drought stress) are two important goals of viticulture, winemaking industry and scientists, especially in a context of climate change (Poni et al., 2018). A promising, efficient and sustainable innovation for the achievement of such objectives could be the use of biostimulants (Rouphael and Colla, 2018, 2020). The definition of biostimulants has been intensively debated over the last decade, mainly for regulatory purposes (du Jardin, 2012, 2015; Yakhin et al., 2017; Caradonia et al., 2019). Recently under the EU fertilizer regulation 2019/1009: a plant biostimulant shall be "a*n EU fertilizing product able to modify plant physiological functions, with the objectives of enhancing one or more of the following agronomic claims: i) tolerance/resistance to abiotic stressors, ii) nutrient uptake and efficiency, iii) qualitative characteristics and iv) availability of nutrients confined in the rhizosphere or to the soil*" (European Union [EU], 2019). The effectiveness of natural plant biostimulants (humic acids, seaweed and plant extracts, protein hydrolyzates, and silicon) in imparting tolerance for horticultural crops (fruit trees, grapevine, and vegetables) against sub-optimal conditions (high temperature and radiation, drought, and biotic pressure), has been attributed to several putative direct and indirect physiological and molecular mechanisms. Among these, there are: 1) enhanced macro and micronutrient uptake due to a modulation of the root system architecture in terms of biomass, soil exploitation, branching and density, 2) increased physiological status (higher leaf CO_2 exchange rates, stomatal conductance, leaf water potential, and water use efficiency), 3) regulation of key genes involved in detoxification process and synthesis of osmolytes (proline, glycinebetaine and sorbitol), and 4) modulation of phytohormone signaling (Mancuso et al., 2006; Calvo et al., 2014; Yakhin et al., 2017; Rouphael and Colla, 2020). Researches on the beneficial use of biostimulants in the fruit production sector, grapevine in particular, are still limited compared to cereals and vegetables, and the findings are not always consistent in terms of repeatability and efficacy (Basile et al., 2020). This evidence is probably associated to the perennial nature of the woody tree species and the variability of the environmental conditions occurring year after year under field conditions.

Despite the fact that several studies regarding the application of biostimulants in the frame of modulating the nutritional, functional and aromatic profile of berries, musts and wines composition have been conducted (Parrado et al., 2007; Ferrara and Brunetti, 2010; Martínez-Gil et al., 2012, 2013; Sánchez-Gómez et al., 2016a,b; Frioni et al., 2018; Popescu and Popescu, 2018), the information on the effects of biostimulant application on grapevine morpho-anatomical, biochemical and physiological response mechanisms to counteract biotic/abiotic stresses is still missing.

The aim of this experiment was to evaluate, in a concurrent climate and regulation change scenario, the application of a plant extract to mitigate possible negative effects due to the

reduced doses of synthetic chemicals, in particular metallic copper, in *Vitis vinifera* L. subsp. *vinifera* "Aglianico." Therefore, four combinations of a tropical plant extract and conventional chemicals for disease control were applied in a vineyard in southern Italy, over two years, and vine response was evaluated in terms of growth performance, morpho-physiological traits and berry quality.

MATERIALS AND METHODS

Plant Material, Cultural Practices and Experimental Design

The experimental trial was conducted in a commercial vineyard (Fonzone-Caccese winery, F-Cw) (40°57′50.0″N 15°03′49.8″E, 400–450 m asl), located in Paternopoli (Avellino, Campania region, Southern Italy). The study was conducted during two consecutive growing seasons 2017–2018 on *Vitis vinifera* L. "Aglianico" (clone VCR 23), grafted onto 420 A (*V. riparia* × *V. berlandieri*). Vines were planted in 2006 on a medium west-faced slope (about 10%), with W-E row direction. A meteorological wireless station (WatchDog, PCE Instruments) was installed at the beginning of 2017 to collect air temperature and rainfall data during the two years of experimental trials.

Along the slope, two different soils (in the upper and lower parts, Haplic Calcisols and Calcaric Cambisols, respectively; IUSS Working Group WRB, 2015) were identified by Brook et al. (2020) through a pedological survey supported by georadar investigations. Although both soils were classified as silt loam, they showed a different hydraulic behavior due to the different presence of sand and rock fragments in their horizons (Brook et al., 2020). Therefore, the experimental trial was realized only on the slope surface characterized by the presence of Calcisol.

Plantation spacing was 2.2 × 1 m, yielding a plant density of 4,500 vines ha^{-1}; vines were trained to a vertical shoot positioned espalier system, spur pruned (horizontal spur cordon at 90 cm height from the ground), with a bud load of about 10 $vine^{-1}$ on 5 spurs. During both years, canopy management operations were performed in terms of suckering (i.e., the mechanical removing of shoots arising from the trunk and from the cordon, except those from spurs) and vertical shoot positioning.

The experimental trials were based on different treatments for disease control management. More specifically, different spraying plant protection mixtures were obtained by the combination of two levels of a copper oxychloride commercial product (Coprantol WG, Syngenta) and only one level of wettable sulfur (Tiovit jet, Syngenta) with or without the addition of a tropical plant extract in liquid formulation (Trym®, Italpollina, Rivoli Veronese, VR, Italy). The trials were conducted on 12 adjacent vine rows (3 rows × 4 disease control treatments) of a vineyard homogeneous for exposition, slope and pedological characteristics. The experimental design compared four treatments: T1, F-Cw industry water mixture of copper oxychloride (thus considered the control treatment), obtained following ordinary dose prescriptions (400 g hL^{-1}) for copper oxychloride and wettable sulfur (400 g hL^{-1}); T2, F-Cw industry water mixture (copper oxychloride at 400 g hL^{-1} + sulfur at 400 g hL^{-1}) plus Trym® (0.5 lt ha^{-1}); T3, F-Cw industry water mixture with halved dose of copper oxychloride (200 g hL^{-1}) and ordinary dose for wettable sulfur (400 g hL^{-1}); T4, F-Cw industry water mixture with halved dose of copper oxychloride (200 g hL^{-1}) and ordinary dose for wettable sulfur (400 g hL^{-1}) plus Trym® (0.5 lt ha^{-1}). The mixtures were sprayed directly on canopy with a shoulder sprayer pump (GeoTech SP 300 4T), equipped with three flat spray nozzles, operating at a pressure of about 15 bar, dispensing up to 10 hL of water solution according to the canopy growth. During both years, six spraying applications were performed, starting from the phenological phase of the third-fourth leaves unfolded (BBCH 13-14) to the berry touch complete (BBCH 79) as reported in Lorenz et al. (1995).

Biostimulant Characteristics

The tropical plant extract biostimulant Trym® was provided by the Italpollina Company (Rivoli Veronese, Italy). Trym® is a commercial plant biostimulant produced through water extraction and fermentation of tropical plant biomass from *Aloe* spp. and *Hybiscus* spp. The final product contains mostly micronutrients, enzymes involved in the activation of natural defense genes (proteases, *N*-acetyl-D-glucosamine kinase, B-glucuronidase, and B-galactosidase), aminoacids, and vitamins. Trym® has a density of 1.023 kg L^{-1}, a pH of 4.1. It contains 0.85 g kg^{-1} of total proteins, 6.22 g kg^{-1} of sugars (3.97 g kg^{-1} of glucose, 2.10 g kg^{-1} of fructose, and 0.15 g kg^{-1} of sucrose) and 1.62 g kg^{-1} of starch.

It contains 221 g kg^{-1} of free amino acids, including 56 g kg^{-1} of essential aminoacids. The aminogram of the product (in g kg^{-1}) is as follows: Ala (0.29), Arg (0.20), Asn (0.12), Asp (0.38), Glu (0.01), Gly (0.10), His (0.14), Ile (0.22), Leu (0.48), Lys (0.14), Orn (2.92) Phe (5.01), Pro (0.32), Ser (0.26), Thr (0.08), Trp (4), Tyr (0.05), and Val (0.23). The total phenolics, determined following the methods reported by Carillo et al. (2019), are 6.51 mg of gallic acid equivalent per gram of f.w. product. The Trym® mineral composition, determined by ion chromatography as described by Cirillo et al. (2019), is as follows (g kg^{-1} f.w): $N–NH_4$ (0.92), $N–NO_3$ (1.92), K (2.71), Na (1.64), Ca (2.86), Mg (2.02), Cl (5.12), and SO_4 (13.30). No detectable phytohormones have been reported in Trym®.

Biometric Analyses and Yield

Biometric analyses were performed during four main phenological phases:pre-flowering, fruit set, veraison and harvest on 15 plants per treatment, selected on the central row of each treatment, in order to avoid drift interference. Two-year-old shoots (holding the production of the year) per plant were selected to monitor growth by recording shoot length, number of leaves and leaf area. Leaf area was estimated by measuring the lamina width and applying the equations calculated based on the measurement of width and area of 25 leaves per treatment by means of a electronic leaf area meter (LI-3100 model, LI-COR Inc., Lincoln, Nebraska, United States), according to Caccavello et al. (2017).

The bud break rate was determined on the selected shoots as the ratio between the number of shoots and buds.

The fruit set was also analyzed on the same shoots. More specifically, all the bunches on the shoots were photographed with a digital camera and images were subjected to digital image analysis through the software Image J (Rasband, NIH) to count the number of visible flowers per bunch. On the same date, from other plants, 12 bunches for treatment were photographed and sampled: for them, the number of flowers per bunch was counted both through digital image analysis and manually to achieve the real flower number. The relationships between the flowers counted through image analysis and the real number of flowers were extracted and the equations used to estimate the real number of flowers per bunch also in all the other bunches. In addition, at the harvest phase, the same procedure was repeated for the berries in order to calculate the fruit set rate as the ratio between the number of berries and of flowers. Finally, at the harvest phase, the number of bunches, their weight (total yield per vine) and the berry diameter (on 150 fruits per treatment) were also recorded. Vine fertility was then estimated as both real fertility (RF, the bunch number per number of buds) and potential fertility (PF, the bunch number per number of shoots.

Leaf Gas Exchange and Chlorophyll "*a*" Fluorescence Emission

Leaf gas-exchange and chlorophyll "*a*" fluorescence emission measurements were carried out on 2 well-exposed and fully expanded leaves per 15 plants during the veraison phase of the two growing seasons (2017–2018). Net CO_2 assimilation rate (P_n) and stomatal conductance (g_s) were performed by means of a portable infra-red gas-analyzer (LCA 4; ADC, BioScientific, Hoddesdon, United Kingdom) equipped with a broad-leaf PLC (cuvette area 6.25 cm^2). Chlorophyll "*a*" fluorescence emission was measured using a portable FluorPen FP100 Max fluorometer with a light sensor (Photon System Instruments, Brno, Czech Republic). A blue LED internal light of 1–2 μmol photons m^2 s^{-1} was used to induce the ground fluorescence F_0 on 30′ dark adapted leaves. A saturating light pulse of 3.000 μmol photons m^2 s^{-1} was applied to induce the maximal fluorescence level in the dark, F_m. The following parameters were considered: the maximum PSII photochemical efficiency (F_v/F_m) calculated as $(F_m - F_0)/F_m$, the quantum yield of PSII linear electron transport (Φ_{PSII}) and non-photochemical quenching (NPQ) (Genty et al., 1989; Bilger and Björkman, 1990). The measurements in the light were conducted from 12:00 to 14:00 pm under environmental Photosynthetic Photon Flux Density (PPFD) ranging between 1,800 and 2,300 photons m^2 s^{-1}.

Leaf Traits and Chlorophyll Quantification

During the growing season 2018, at the veraison phase, leaf functional traits, namely specific leaf area (SLA), leaf dry matter content (LDMC), and relative water content (RWC) were determined on 10 well-exposed and fully expanded leaves per treatment, following the methods reported in Cornelissen et al. (2003). The SLA was calculated as the ratio between leaf area and leaf dry mass (cm^2 g^{-1}). LDMC was measured as the leaf oven-dry mass (at 75°C for 48 h) divided by its water-saturated fresh mass and expressed as g g^{-1} wslm (water-saturated leaf mass). The RWC was expressed as percentage of (fresh weight − dry weight)/(saturated weight − dry weight). The saturated fresh weight was measured submerging the petiole of leaf blades in distilled water for 48 h in the dark.

From the same plants, five leaves per treatment were collected and used for the extraction of chlorophylls and carotenoids. Pigments were extracted in ice-cold 100% acetone with a mortar and pestle and centrifuged at 5,000 rpm for 5 min (Labofuge GL, Heraeus Sepatech, Hanau, Germany). The absorbance of supernatants was quantified by a spectrophotometer (UV-VIS Cary 100, Agilent Technologies, Santa Clara, CA, United States) at wavelengths of 470, 645, and 662 nm. The pigment content was calculated according to Lichtenthaler (1987) and expressed in μg cm^{-2}.

Functional Anatomical Traits in Leaves and Fruits

During the growing season 2018, at the veraison phase, five fully expanded leaves were sampled from five plants per treatment. Each leaf was dissected to obtain two sub-samples from the median region of the lamina: one devoted to thin sectioning, the other to peeling for stomata characterization. The subsamples were immediately submerged in the FAA chemical fixative (5 mL 40% formaldehyde, 5 mL glacial acetic acid, and 90 mL 50% ethanol) for several days. During the harvest phase, five berries from five plants per each treatment were collected by dissecting them directly on the plant in order to analyze fruit samples having the same exposition to the light. Fruit samples were fixed in FAA as well. The sampled leaves and berries were further dissected under a reflected light microscope (SZX16, Olympus, Hamburg, Germany) to obtain subsamples of leaves (5 × 6 mm) and berries (8 × 8 mm, removing the seeds) which were dehydrated in an ethanol series (up to 95%), infiltrated and embedded in the JB4® acrylic resin (Polysciences, United States). Cross sections of the leaf lamina and longitudinal sections of the fruits were cut by means of a rotary microtome at 5 μm thickness. Leaf sections were stained with 0.5% toluidine blue in water (Feder and O'Brien, 1968), mounted with mineral oil for microscopy, and observed under a transmitted light microscope (BX60, Olympus, BX 60). Fruit sections were mounted, unstained, with mineral oil for fluorescence microscopy and observed under an epi-fluorescence microscope (BX60, Olympus) equipped with a mercury lamp, band-pass filter of 330–385 nm, dichromatic mirror of 400 nm and above, and a barrier filter of 420 nm and above, for the observation of simple phenolic compounds and suberized/lignified cell walls (Fukazawa, 1992; Ruzin, 1999; De Micco and Aronne, 2007). Images of leaf and fruit sections at different magnifications were captured though a camera (CAMEDIA C4040, Olympus) and analyzed through the image analysis software Olympus AnalySIS 3.2, in order to quantify the following morpho-anatomical traits: the thickness of the

leaf lamina (total leaf thickness, TLT), of the palisade and spongy parenchyma (palisade tissue thickness, PT and spongy tissue thickness, ST); the ratio between the thickness of the palisade parenchyma and the thickness of the entire foliar lamina (PT/TLT); the ratio between the thickness of the spongy parenchyma and the thickness of the entire foliar lamina (ST/TLT); the percentage of intercellular spaces per surface area in the spongy parenchyma (intercellular spaces, IS); the thickness of the collenchyma located in the subepidermal portions of the upper (TCU) and lower (TCL) lamina surfaces at the vein level.

As regards the quantification of stomata traits, the abaxial epidermis was carefully peeled off with a tweezer, and the epidermis strips were flattened and mounted on a glass slide with distilled water. Three film strips from each sample were observed under a transmitted light microscope (BX60, Olympus, Hamburg, Germany). Digital images of the epidermis were collected and analyzed as reported above to measure: stomata frequency (SF), calculated by counting the number of stomata in five regions of the epidermis and expressed as the number of stomata per mm^2; the guard cell length (GCL), quantified by measuring the length pole to pole, and the guard cell width (GCW) in the median position, in 20 stomata per sample.

Leaf Mineral Composition

During both growing seasons, at the veraison phase, six fully expanded leaves per treatment were sampled. Leaf dry tissues were finely ground with a mill (IKA, MF10.1, Staufen, Germany) with 0.5 mm-sieve. For the evaluation of mineral leaf composition in terms of cations (Na, NH_4, K, Mg, and Ca), anions (NO_3, SO_4, PO_4, and Cl) and organic acids (malate, tartrate, citrate, and isocitrate), 250 mg of dried material were suspended in 50 mL of ultrapure water (Milli-Q, Merk Millipore, Darmstadt, Germany), freezed and subjected to 10 min shaking in a water bath (ShakeTemp SW22, Julabo, Seelbach, Germany) at 80°C. Anions and cations were separated and quantified by ion chromatography equipped with a conductivity detection (ICP 3000 Dionex, Thermo fisher Scientific Inc., MA, United States), according to Zhifeng and Chengguang (1994).

Fruit Quality Traits

During both growing seasons, at the harvest phase, analytical determinations of standard chemical parameters, namely soluble solids content (SSC), juice pH and titratable acidity (TA), were carried out on a sample of 10 berries per each of 15 plants per treatment. To ensure a representative sample, berries were picked from the top to the bottom and from the internal to the external part of the bunch. The SSC was determined by refractometric analysis (HI96801 digital refractometer, HANNA Instruments Italia Srl, Padua) on unfiltered juice, obtained by squeezing the berries (European Union [EU], 1990), and expressed in Brix. The remaining juice was filtered and diluted 1:1 in distilled water and used to measure the juice pH and the TA. Juice pH values were recorded using a digital pH meter (CLB22, Crison Instruments, Alella, Barcelona, Spain); whereas for TA determination, diluted sample were titrated with a 0.1 N NaOH solution up to pH 8.2 and the titratable acidity was expressed as g L^{-1} of tartaric acid equivalent.

Statistical Analysis of Data

All experimental data were analyzed with the SPSS 13 statistical software (SPSS Inc., Chicago. IL, United States). The growth data were analyzed by three-way analysis of variance (ANOVA) considering the year (Y), the foliar treatment (T) and the phenological phase (PP) as main factors. A two-way ANOVA was performed on data collected at fruit set for shoot fertility and fruit set, and at harvest for yield components (total bunch weight and the number of bunches per vine) and for main qualitative parameters of berries (average berry diameter, SSC, pH, and TA), considering the year (Y) and the foliar treatment (T) as main factors. Whenever the interactions were significant, a one-way ANOVA was performed. Leaf traits and anatomical data were subjected to one-way ANOVA. To separate treatments per each measured parameter, the Duncan's multiple range test was performed. The verification of normality was performed through the Shapiro–Wilk test; the percentage data were previously subjected to arcsine transformation.

RESULTS

Meteorological Course

The montly average mean air temperature was similar during the two growing seasons, with an annual mean temperature of 14.2 and 14.5°C in 2017 and 2018, respectively (**Figure 1A**). The hottest month was August in 2017 (monthly average mean temperature of 26.2°C; maximum temperature of 34.9°C), while July in 2018 (monthly average mean temperature of 23.8°C; maximum temperature of 30.9°C). The cumulative annual precipitation was 568 mm in 2017, while 955 mm in 2018. The monthly precipitation of July and August was of 6.3 and 0.5 mm, respectively in 2017, while it was of 7.6 and 110.4 in 2018. Therefore, during the second year of trial the calculated difference between monthly rainfall and ET_0 indicates a lower water availability during the grapevine growing season in 2017 (**Figure 1B**).

Growth Analysis and Production

Growth parameters (bud break percent, shoot length, number of leaves, and leaf area per shoot) of the four treatments applied to Aglianico vines, measured at the four phenological phases during the two growing seasons, are reported in **Table 1**. Considering the effects of year as main factor, results showed that in 2018, all growth parameters were significantly higher than 2017. Concerning the foliar treatment, the T4, containing Cu at 50% and Trym®, caused a significant reduction of all growth parameters but bud break percent. In particular, the average shoot length was reduced by 17% compared to T1 (Cu at 100%) and by about 9% compared to both T2 (Cu at 100% and Trym®) and T3 (Cu at 50%) (**Table 1**). Similarly, the total leaf area and leaf number per shoot were significantly decreased by T4 application compared to the other treatments (**Table 1**).

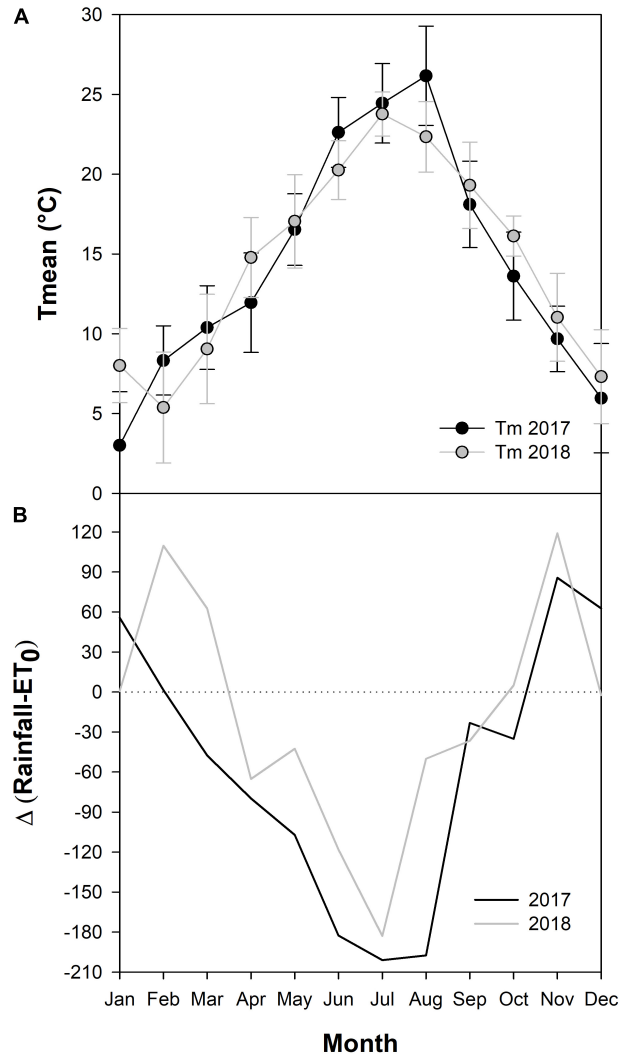

FIGURE 1 | Diagrams showing the monthly average mean air temperature **(A)** and water deficit (calculated as difference between rainfall and ET0) **(B)** for 2017 (black line) and 2018 (gray line).

Overall, among the vegetative phases, the major increment of growth parameters was recorded at veraison. The interaction between year and foliar treatment (Y × T) was significant for the average shoot length, number of leaves, and total leaf area (**Table 1**). In detail, the highest shoot length was observed in T1 during the year 2018; moreover, this parameter resulted significantly lower in all the other treatments both in 2017 and in 2018, with the lowest value in T4 in 2017 (**Figure 2A**). Similarly, the T4 treatment in 2017 induced the lowest leaf number and leaf area per shoot, whereas T2, the treatment containing the same dose of Cu but added with Trym®, showed the highest values (**Figures 2B,C**).

Significant differences were also found in grapevine production components (**Table 2**). Compared to 2017, in 2018 there was a decrease in potential fertility (−13.3%) and in the number of bunches per vine (−41%), and a contemporary increase in the fruit set (+19.4%) and in the average diameter of berries (+28.1%). The real fertility and weight of bunches per plant, however, were not significantly influenced (**Table 2**).

Fruit set was affected by the Y × T interaction, with foliar treatment T1 and T2 in 2018 resulting in 54 and 53% of fruit set, followed by T2 (45%) and T1 (39%) in 2017 and T4 (44%) in 2018 as intermediate values, finally by T3 (28.5%) and T4 (26.5%) in 2017 and T3 (16%) in 2018.

The Y × T interaction resulted significant in all the main components of the yield, since the bunch number per vine was generally higher during 2017, with the highest number of bunches obtained in T1 treatment, whereas the lowest values were recorded in T2 and T4 treatments of 2018 (**Figure 3A**). On the contrary, the T1 treatment in 2018 induced the highest yield in terms of total bunch weight (nearly 1 kg per vine) and the T4 treatment in 2017 showed the lowest yield harvested (**Figure 3B**). Furthermore, even though in general the berry size resulted significantly increased in all the treatments in 2018, the T4 treatment in 2018 showed the highest value and the overall lowest one in 2017 (**Figure 3C**). Finally, SSC of berries was highest in T1 and T3 in 2017, intermediate in T2 and T4 in 2017 and T1 in 2018 and reached the lowest level in T2, T3, and T4 in 2018 (**Figure 3D**).

TABLE 1 | Main effects of year, foliar treatment and phenological phase on vine bud break, shoot length, number of leaves per shoot, total leaf area per shoot of *V. vinifera* "Aglianico" vines. Mean values and significance of main factors' interactions are shown. Different letters within column indicate significant differences according to Duncan's multiple-range test ($P \leq 0.05$).

	Bud break (%)	Shoot length (cm)	Leaves (n shoot^{-1})	Total leaf area (cm^2 shoot^{-1})
Year (Y)				
2017	92.70^b	82.09^b	25.38^b	1,584.52^b
2018	99.50^a	110.18^a	44.69^a	3,287.56^a
Foliar treatment (T[1])				
T1	95.60^a	105.41^a	36.17^b	2,482.02^a
T2	99.30^a	95.91^b	39.22^a	2,653.31^a
T3	94.20^a	95.62^b	36.08^b	2,537.06^a
T4	95.20^a	87.60^c	28.70^c	2,071.78^b
Phenological Phase (PP)				
Pre-flowering	*n.a.*	80.08^b	26.74^b	1,960.70^c
Fruit set	*n.a.*	104.60^a	43.21^a	2,954.23^b
Veraison	*n.a.*	100.70^a	46.20^a	3,312.91^a
Harvest	*n.a.*	99.05^a	24.01^b	1,516.32^d
Significance[2]				
Y	***	***	***	***
T	NS	***	***	***
PP	*n.a.*	***	***	***
Y × T	NS	***	***	**
Y × PP	*n.a.*	NS	***	***
T × PP	*n.a.*	NS	NS	NS

[1]T1 – only industry water mixture of copper oxychloride; T2 – industry water mixture of copper oxychloride plus Trym®; T3 – half dose of copper oxychloride water mixture; T4 – half dose of copper oxychloride water mixture plus Trym®.
*[2]NS, *, **, ***: Non-significant or significant at $P \leq 0.05, 0.01, 0.005$, respectively.*

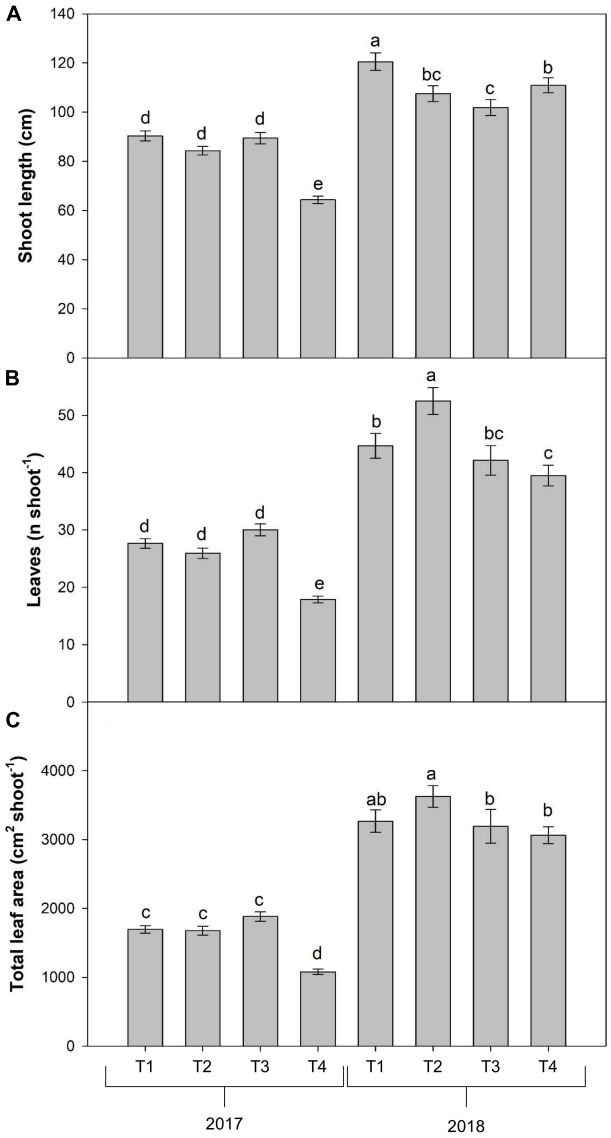

FIGURE 2 | Combined effects of year and foliar treatments shoot length **(A)**, leaf number **(B)** and total leaf area **(C)** per shoot of *V. vinifera* "Aglianico" vines (mean ± SE). Different letters indicate significant differences according to Duncan's multiple-range test ($P \leq 0.05$). [T1 – only industry water mixture of copper oxychloride; T2 – industry water mixture of copper oxychloride plus Trym®; T3 – half dose of copper oxychloride water mixture; T4 – half dose of copper oxychloride water mixture plus Trym®].

Leaf Gas Exchange, Chlorophyll Fluorescence Emission and Photosynthetic Pigment Quantification

With the exception of stomatal conductance (gs), which was affected by the year only (with the highest values recorded in 2018), the physiological data at veraison, in particular the net photosynthetic rate (Pn) and the transpiration rate (E), were influenced by the interaction Y × T (**Table 3**). Indeed, Pn reached the highest level in T2 in 2018 and the lowest in T2 in 2017, whereas all the other treatments were intermediate (**Figure 4A**). Differently, the highest value of transpiration rate was found in T3 and T2 of year 2017 (3.63 and 3.28 mol H_2O $m^{-2}s^{-1}$, respectively), whereas all the treatments in 2017 reached the lowest values (on average 0.93 mol H_2O $m^{-2}s^{-1}$) (data not shown).

The maximum PSII photochemical efficiency (F_v/F_m) was significantly affected by the interaction Y × T, whereas the quantum yield of Φ_{PSII} and NPQ were mainly influenced by the year, with all parameters in 2018 reaching higher values than in 2017, and by foliar treatment (**Table 3**). For instance, the lowest values of F_v/F_m were recorded in T1 and T3 treatments vines during the year 2017 (**Figure 4B**). T2 and T4 elicited significant increase (12.4 and 8.8%, respectively) in Φ_{PSII} compared to the treatments without Trym® (T1 and T3), whereas the NPQ resulted significantly lowered in the foliar treatments containing Trym®, such as T2 and T4 (**Table 3**).

The total chlorophyll and carotenoid contents also increased in 2018 compared to 2017 and were affected by the Y × T interaction (**Table 3** and **Figures 4C,D**).

Indeed, vines treated with sprays containing Cu and Trym® (T2 and T4) limitedly to 2018 also showed a significant rise in the photosynthetic pigment content compared to the other treatments.

Leaf Traits and Functional Anatomical Traits in Leaves and Fruits

As reported in **Table 4**, leaves subjected to the 50%Cu dose showed a significant reduction in the RWC compared to leaves treated with 100% Cu dose, whereas no significant differences were found in SLA and LDMC.

Microscopy observations of leaves and fruits showed that there were no treatment-induced qualitative alterations in the tissue organization (**Figure 4**). The thickness of lamina, of palisade and spongy parenchyma tissues, as well as the spongy parenchyma density (i.e., percent of intercellular spaces) were not influenced by the foliar treatments (**Table 4**). Stomata traits, either frequency or size, were not significantly influenced as well (**Table 4**). The sole leaf anatomical parameter significantly influenced by the foliar treatments was the thickness of the collenchyma layers under the upper epidermis, in correspondence of the veins. This trait showed the lowest value in T1, significantly increased in T3 and T4 which in turn presented significantly lower values than T2 (**Table 4**).

Regarding the fruits, epi-fluorescence microscopy showed an intrinsic fluorescence, due to the presence of waxy substances on the surface of the exocarp, and of phenolic compounds both in vacuoles and along the membranes. The yellow-orange autofluorescence of these phenolic compounds was stronger at the subepidermal layers of cells compared to the inner parenchyma cell layers of the flesh. Indeed, the autofluorescence gradually faded moving toward the inner layers of the flesh. The intensity of autofluorescence was significantly affected by the foliar treatments. In particular, the reduction in the copper dose in T3 and T4 led to an increase in the intensity of phenolic autofluorescence especially in the cells of the sub-epidermal

TABLE 2 | Main effects of year and foliar treatment on potential and real fertility of shoots, fruit set, bunch weight and number, berry diameter, berry juice soluble solids content (SSC), pH, and titratable acidity (TA) of *V. vinifera* "Aglianico" vines. Mean values and significance of main factors' interactions are shown. Different letters within column indicate significant differences according to Duncan's multiple-range test ($P \leq 0.05$).

	Potential fertility	Real fertility	Fruit set (%)	Bunch weight (g vine^{-1})	Bunch number (no. vine^{-1})	Berry diameter (cm)	SSC (°Brix)	pH	TA (g l^{-1} tartaric acid equivalent)
Year (Y)									
2017	1.1 5^{a}	1.07^{a}	34.90^{b}	0.597^{a}	10.64^{a}	1.14^{b}	23.58^{a}	3.16^{a}	7.19^{b}
2018	0.91^{b}	0.97^{a}	41.70^{a}	0.675^{a}	6.28^{b}	1.46^{a}	22.51^{b}	3.25^{a}	7.70^{a}
Foliar treatment (T)[1]									
T1	1.01^{a}	1.03^{a}	46.50^{a}	0.835^{a}	9.33^{a}	1.29^{b}	23.48^{a}	3.19^{a}	7.65^{a}
T2	1.11^{a}	1.13^{a}	49.30^{a}	0.680^{b}	8.77^{a}	1.30^{b}	22.74^{b}	3.27^{a}	7.71^{a}
T3	1.01^{a}	0.97^{a}	22.30^{c}	0.683^{b}	8.50^{a,b}	1.33^{a}	23.17^{a}	3.14^{a}	7.44^{a,b}
T4	0.98^{a}	0.94^{a}	35.10^{b}	0.347^{c}	7.23^{b}	1.29^{b}	22.81^{b}	3.22^{a}	6.98^{b}
Significance[2]									
Y	**	NS	***	NS	***	***	***	NS	*
T	NS	NS	***	***	*	***	***	NS	*
Y × T	NS	NS	***	**	*	***	***	NS	NS

[1]T1 – only industry water mixture of copper oxychloride; T2 – industry water mixture of copper oxychloride plus Trym®; T3 – half dose of copper oxychloride water mixture; T4 – half dose of copper oxychloride water mixture plus Trym®.
[2]NS,, **, ***: Non-significant or significant at $P \leq 0.05, 0.01, 0.005$, respectively.*

layers if compared to T1 and T2 (**Figures 5E,F**). T4 fruits showed not only the highest autofluorescence but also a thicker zone showing such an autofluorescence moving toward the inner part of the flesh (data not shown).

Leaf Mineral Composition

Leaf mineral composition in the first year of trial (2017) showed higher values of Na^+, tartrate and citrate compared to 2018. However, in 2018 PO_4^{3-} increased, while no differences were assessed for NH_4^+ between the two years (**Table 5**). The foliar spray treatment as main factor induced no significant differences for Na^+, NH_4^+, PO_4^{3-} and tartrate, whereas T2 and T4 affected positively the citrate concentration in the leaves compared to the two control treatments without Trym®, such as T1 and T3 (**Table 5**).

The Y × T interaction influenced significantly K^+, Mg^{2+}, and Ca^{2+} which increased in the leaves of vines treated with the foliar spray containing Cu at 50% and Trym® in the year 2018 (**Figures 5A–C**). On the other hand, the nitrate content resulted highest in T1 treatment in 2018 and reached the lowest values in all the other treatments in the same year, whereas it was increased in both the treatments containing the Trym® (T2 and T4) compared to related controls (T1 and T3) in the year 2017 (**Figure 5D**). The concentration of sulfate was generally decreased in all the treatments of 2018 compared to 2017, with the lowest level in T3 (**Figure 5E**). Finally, also the concentration of malate resulted significantly increased in the treatments T3 and T4 of 2018, while no differences were detected among all the other treatments (**Figure 5F**).

Fruit Quality Traits and Mineral Composition

Considering the year as main factor, 2018 showed a decrease in the SSC and an increase in titratable acidity (TA), while the pH was not influenced (**Table 6**). The TA was the only parameter significantly influenced by the dose of copper, showing a decrease in response to 50% Cu. Furthermore, TA in 2017 showed no significant differences among the four treatments. In 2018, however, it decreased in T4 (**Figure 6**). Trym® application, caused a reduction in the SSC compared to T1, while the other parameters were not affected.

Except for the concentration of potassium, the mineral composition of berries was affected as main factors by year and by foliar treatment (**Table 6**). In particular, in the second year of measurements (2018) an increase in the content of NH_4^+, PO_4^{3-}, malate, and a decrease in Mg^{2+}, NO_3^-, Cl^-, tartrate, was recorded compared to the first year (2017) (**Table 6**). Concerning the foliar treatment as the main factor, the reduction of the copper content to 50% led to an increase in Na^+ and PO_4^{3-} when applied alone (T3 vs T1) and to a decrease in NH_4^+ and tartrate content when applied with Trym® (T4 vs T2) (**Table 6**).

DISCUSSION

In this study, we demonstrated that the reduction of Cu application coupled with the distribution of a tropical plant extract through foliar spraying in grapevine can be a promising strategy to support plant protection respectfully of chemical control restrictions. The application of plant-based biostimulants, in grapevine is increasing and has been proven to influence many plant processes, including nutrient absorption, photosynthesis, mechanisms of defense against biotic and abiotic stresses (Gutiérrez-Gamboa et al., 2019). Indeed, the effects are strictly dependent on chemical composition, dose, time of distribution, and cultivar, often leading to distinct plant responses such as increasing or decreasing vegetative growth and photosynthesis (Salvi et al., 2016; Gutiérrez-Gamboa et al., 2019). In the case of Aglianico, the application of the halved dose of Cu together with the tropical plant extract

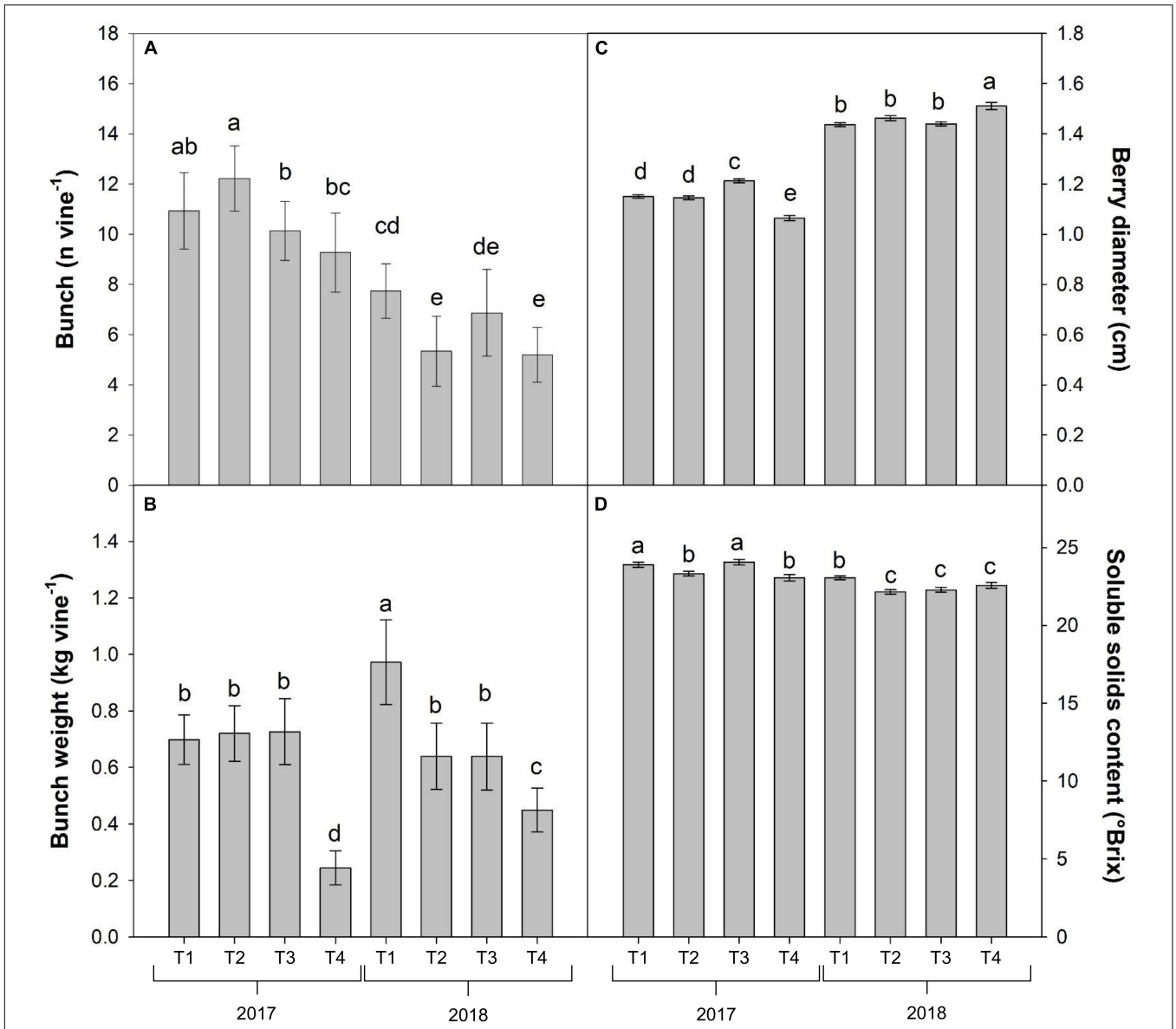

FIGURE 3 | Combined effects of year and foliar treatments on bunches number **(A)** and weight **(B)** per vine and berry diameter **(C)** and soluble solids content **(D)** in *V. vinifera* "Aglianico" vines (mean ± SE). Different letters indicate significant differences according to Duncan's multiple-range test ($P \leq 0.05$). [T1 – only industry water mixture of copper oxychloride; T2 – industry water mixture of copper oxychloride plus Trym®; T3 – half dose of copper oxychloride water mixture; T4 – half dose of copper oxychloride water mixture plus Trym®].

allowed maintaining a reduced vegetative vigor, only partly due to decreased photosynthetic levels, at least compared to the treatment with full Cu dose plus the Trym®. The reduction in vegetative growth, in agreement with other studies, is a desirable trait in Aglianico that is a cultivar characterized by high vigor thus being high-demanding in terms of canopy management (Bavaresco et al., 2005). Interestingly, the reduced dose of Cu determined a significant reduction in the fruit set that was partially recovered by the application of the Trym®. This suggests that the tropical plant extracts could have helped a different allocation of resources, still high due to high photosynthetic efficiency.

It is noteworthy that the application of the different treatments has induced a diverse regulation of plant photosynthetic capacity, determining an adaptation of grapevines to the new environmental conditions. The improvement in photosynthetic performance was evident only in the second year of the treatments. In the first year, the lower levels of gas exchanges (Pn and gs and photosynthetic pigments were not accompanied by any decrease in photochemistry when the tropical plant extract was applied. Conversely, in the second year, the addition of Trym® significantly improved leaf gas exchanges and photochemistry as well as the synthesis of photosynthetic pigments. The differential responses observed in the two years,

TABLE 3 | Main effects of year and foliar treatment, on net photosynthetic rate (Pn), stomatal conductance (gs), leaf transpiration rate (E), maximum quantum efficiency of PSII photochemistry (F_v/F_m), quantum yield of PSII linear electron transport (Φ_{PSII}), non-photochemical quenching (NPQ) and leaf pigment quantification of *V. vinifera* "Aglianico" vines at veraison. Mean values and significance of main factors' interactions are shown. Different letters within column indicate significant differences according to Duncan's multiple-range test ($P \leq 0.05$).

	Pn (μmol m^{-2} s^{-1})	gs (mmol m^{-2} s^{-1})	E (mol H_2O m^{-2}s^{-1})	F_v/F_m	ΦPSII	NPQ	Total chlorophyll content (μg cm^{-2})	Total carotenoids content (μg cm^{-2})
Year (Y)								
2017	2.90^{b}	17.63^{b}	0.93^{b}	0.758^{b}	0.328^{b}	1.38^{a}	36.79^{b}	7.68^{b}
2018	9.43^{a}	73.18^{a}	3.17^{a}	0.797^{a}	0.395^{a}	1.23^{b}	62.21^{a}	15.14^{a}
Foliar treatment (T[1])								
T1	5.85^{b}	45.53^{a}	1.83^{b}	0.770^{b}	0.346^{c}	1.52^{a}	44.20^{c}	10.43^{c}
T2	6.97^{a}	44.96^{a}	2.04^{a,b}	0.785^{a}	0.389^{a}	1.15^{c}	53.63^{a}	11.99^{b}
T3	5.99^{b}	50.74^{a}	2.26^{a}	0.770^{b}	0.340^{c}	1.36^{b}	47.46^{b,c}	9.19^{c}
T4	5.86^{b}	40.41^{a}	2.06^{a,b}	0.786^{a}	0.370^{b}	1.13^{c}	52.70^{a,b}	14.04^{a}
Significance[2]								
Y	***	***	***	***	***	**	***	***
T	***	NS	*	***	***	***	***	***
Y × T	***	NS	*	***	NS	NS	***	***

[1]T1 – only industry water mixture of copper oxychloride; T2 – industry water mixture of copper oxychloride plus Trym®; T3 – half dose of copper oxychloride water mixture; T4 – half dose of copper oxychloride water mixture plus Trym®.
[2]NS,, **, ***: Non-significant or significant at $P \leq 0.05, 0.01, 0.005$, respectively.*

may be ascribed to the different climatic conditions especially regarding the water deficit intensity and duration throughout the growing season. Indeed, the improvement in all the morphological and physiological traits recorded in the second year of the trial, especially with the application of Trym®, might be due to the higher cumulated rainfall compared to the previous year. Another explanation may be that many critical physiological processes, such as nutrient uptake by soil and photosynthetic carbon assimilation, need more time to be influenced, and that the plant extract exerts a long-lasting effect on the photosynthetic capacity. Even if plants did not show stress signals, as indicated by the comparable F_v/F_m ratio, the prolonged application of Trym® on leaves for two consecutive years might have promoted the PSII electron transport activity, allocating the reductive power of the electron transport chain in carbon fixation, rather than in non-radiative dissipation mechanisms. This assumption is confirmed by the higher Pn rates and lower NPQ index in plants treated with Trym® and both levels of copper oxychloride compared to plants without the Trym® application, during the second year of treatments. The positive effects exerted by the tropical plant extract on gas exchanges and photochemistry in the second year of treatment may also be ascribed to the enhancement of some leaf functional traits. More specifically, the increase in total leaf area, as well as the high content of chlorophylls and carotenoids found in plants treated with vegetal-based plant biostimulant, may have helped the light interception and conversion by the photosynthetic apparatus, thus favoring the photosynthesis. A direct presumed mechanism in the rise of photosynthesis could be attributed to the increase in N assimilation in crops, due to the positive effects of signaling molecules (amino acids and soluble peptides present in the product) on the production of C skeletons and energy supply, which are needed for amino acid biosynthesis (Colla et al., 2015, 2017). Moreover, another putative indirect mechanism behind the biostimulant activity (especially in T4) of tropical plant extract is the modulation of the root system architecture (e.g., length, number, density, and expansion of lateral roots) thus improving nutrient uptake (higher K, Ca, and Mg concentration in leaf tissue). Particularly, Mg is an essential element for plant growth and/or development and is involved in a wide range of biochemical and physiological activities, including pigment synthesis and photosynthetic carbon fixation (Gransee and Führs, 2013; Kumar et al., 2015). It is well known that the addition of copper-based foliar fertilizer increases photosynthetic pigment content compared to untreated samples by improving of soil chemical properties (Zhu et al., 2012). In our case, the treatment obtained mixing a half dose of copper oxychloride with Trym® did not produce a stimulatory effect on photosynthetic performance, despite the increased pigment content, probably because the chosen dose is not suitable for grapevine plants at veraison stage. However, the gas exchanges and photochemical behavior are also dependent on changes in plant structural traits induced by the different treatments. Hence, it cannot be excluded that the harmonization of structural and functional plant traits at the whole plant level has determined the overall grapevine physiological response.

The lack of any changes in the mesophyll and stomata traits related to the control of water conductivity and gas-exchange control, suggests that the treatments did not induce permanent structural changes in leaves, but adaptation relies more on short-term physiological adjustment.

Furthermore, the application of the tropical plant extracts, although slightly reducing yield compared to the normal farm practice, allowed reducing the SSC in grapes while maintaining a satisfying level of titratable acidity and also acting on berry size, content of some minerals and histological traits. In grapevine, it has been reported that foliar application of seaweed extracts induced a raise in uptake of cations, such as potassium and calcium that turned in an increased vegetative

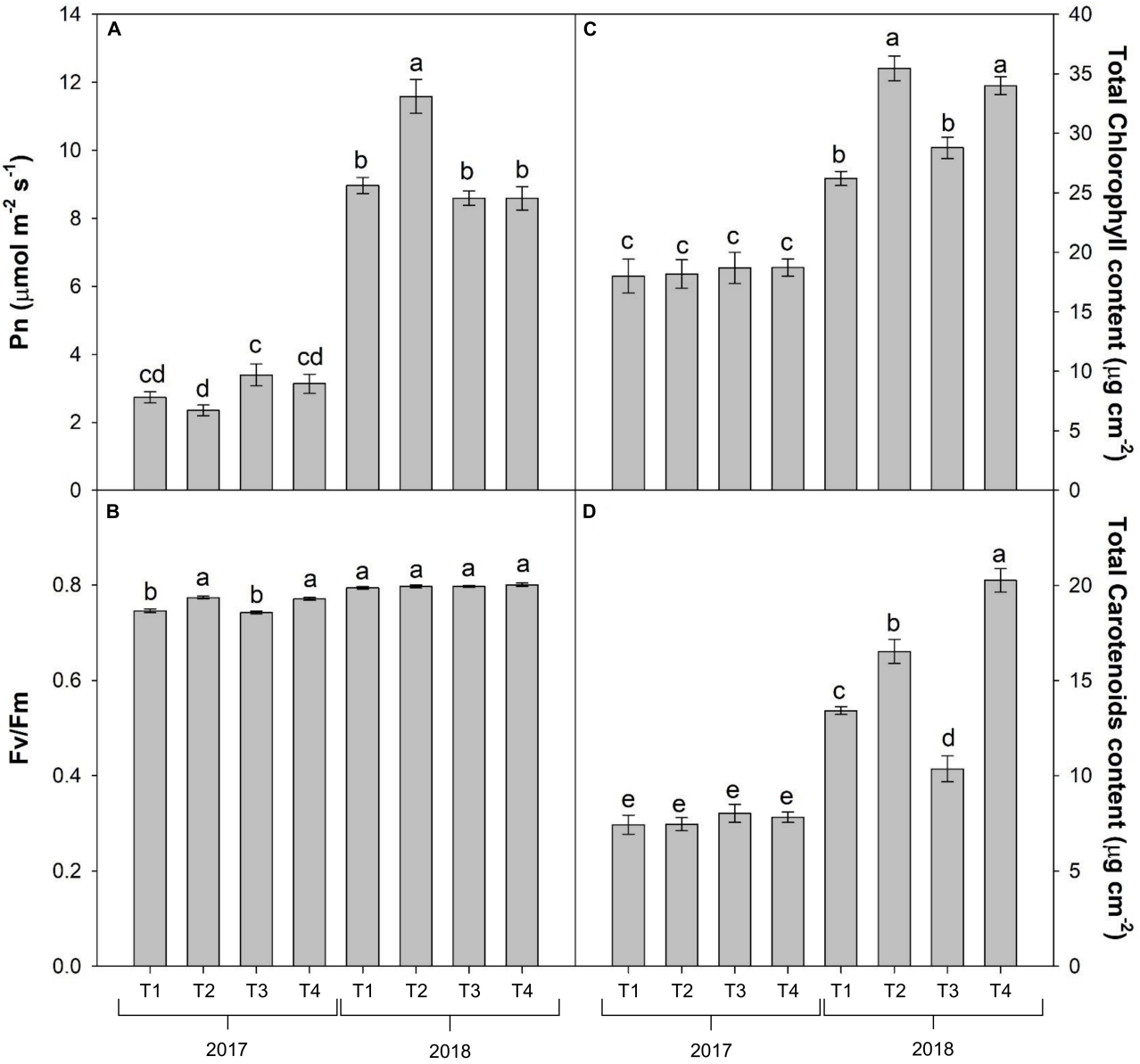

FIGURE 4 | Combined effects of year and foliar treatments on net photosynthetic rate (P_n) **(A)**, maximum quantum efficiency of PSII photochemistry (F_v/F_m) **(B)**, total chlorophyll content **(C)** and total carotenoids content **(D)** of *V. vinifera* "Aglianico" vines (mean ± SE). Different letters indicate significant differences according to Duncan's multiple-range test ($P \leq 0.05$). [T1 – only industry water mixture of copper oxychloride; T2 – industry water mixture of copper oxychloride plus Trym®; T3 – half dose of copper oxychloride water mixture; T4 – half dose of copper oxychloride water mixture plus Trym®].

growth (Mancuso et al., 2006), In the present experiment, despite increased leaf concentrations of potassium, magnesium and calcium were observed when Trym® was added to the halved Cu dose, our data indicate a reduction in average shoot length whenever the tropical plant extract was applied independently of the copper dose. A higher level of magnesium in leaves has been suggested to counteract the incidence of bunch stem necrosis, that is a well-known physiological disorder in grapevine, related to magnesium deficiency (Bondada and Keller, 2012). As regards histological traits, the half dose of Cu together with the tropical plant extract lead to an increased autofluorescence of phenolics in the subepidermal layers and also an increase in the thickness of the flesh containing such phenolics, likely indicating an augmented content in phenolics in berries. This suggests a redirection of plant resources toward the production of secondary metabolites, whose accumulation is a typical strategy adopted by plants to improve the physiological defenses against abiotic and biotic stresses. Indeed, phenolic compounds are produced through the phenylpropanoid pathway which is soon activated to mediate plant interaction with abiotic and biotic factors and is considered the key of the robustness of gymnosperms and angiosperms to

TABLE 4 | Main effects of foliar treatments on functional anatomical traits in leaves of *V. vinifera* "Aglianico" vines. Different letters within column indicate significant differences according to Duncan's multiple-range test ($P \leq 0.05$).

	SLA ($cm^2\ g^{-1}$)	LDMC ($g\ g^{-1}$)	RWC (%)	TLT (μm)	PT/TLT (μm)	ST/TLT (μm)	IS (%)	TCU (μm)	TCL (μm)	SF (mm^2)	GCL (μm)	GCW (μm)
Foliar treatment (T^1)												
T1	147.12^a	0.242^a	91.95^a	185.61^a	0.450^a	0.350^a	34.75^a	171.19^c	91.57^a	218.60^a	61.40^a	11.73^a
T2	160.39^a	0.236^a	89.35^a	177.31^a	0.447^a	0.358^a	35.48^a	208.44^a	114.29^a	274.27^a	54.40^a	10.20^a
T3	156.27^a	0.237^a	90.14^a	189.46^a	0.447^a	0.367^a	37.97^a	190.83^b	94.13^a	238.97^a	51.87^a	8.93^a
T4	144.26^a	0.244^a	82.12^b	177.77^a	0.447^a	0.358^a	39.84^a	190.48^b	112.05^a	300.41^a	47.00^a	14.60^a
Significance^2												
T	NS	NS	*	NS	NS	NS	NS	***	NS	NS	NS	NS

[1] T1 – only industry water mixture of copper oxychloride; T2 – industry water mixture of copper oxychloride plus Trym®; T3 – half dose of copper oxychloride water mixture; T4 – half dose of copper oxychloride water mixture plus Trym®.

*[2] NS, *, **, ***: Non-significant or significant at $P \leq 0.05, 0.01, 0.005$, respectively.*

SLA, specific leaf area; LDMC, leaf dry matter content; RWC, relative water content; TLT, total leaf thickness; PT/TLT, ratio between the thickness of the palisade parenchyma and the thickness of the entire foliar lamina; ST/TLT, ratio between the thickness of the spongy parenchyma and the thickness of the entire foliar lamina; IS, percentage of intercellular spaces per surface area in the spongy parenchyma; TCU and TCL, thicknesses of the collenchyma located in the subepidermal portions of the upper and lower lamina at the vein level; SF, stomata frequency; GCL, guard cell length; GCW, guard cell width.

cope with stresses (Vogt, 2010). The biostimulatory action of the tropical plant extract on the synthesis and accumulation of antioxidant molecules (phenolic compounds) was likely related to the activation of secondary metabolism, in particular the increase in gene expression of the phenylalanine (tyrosine) ammonia-lyase enzyme, involved in the phenylpropanoid pathway (Schiavon et al., 2010; Ertani et al., 2011). Phenolics are generally accumulated in various plant tissues, especially in the subepidermal layers of organs, in order to protect them by predators and pathogens, being non-lethal feeding deterrents. Their localization at the periphery of plant organs enhances their role in chemical protection given that any injury at the tissue surface would cause the prompt release of the phenolics stored in the cells, thus likely activating inducible defenses (Franceschi et al., 1998; Graham et al., 2004). In the current experiment, the distribution of the tropical plant extract would likely have not only a role in berry defense, but is also a positive trait for the achievement of the phenolic maturity. Indeed, the optimal grape maturity is cultivar specific and defined by a specific combination of three main factors: i) technological maturity (i.e., sugar, acids or their ratio); ii) phenolic maturity (i.e., quantity and quality of all tannins and pigments); iii) aromatic ripeness (i.e., typical olfactory features reached without appearance of untypical aging or excessive veggie-green aromas). The decoupling between the above three factors is strongly aggravated under a global warming scenario (Palliotti et al., 2014). Higher temperatures increase the speed of sugar accumulation, hasten acid degradation, alter flavor compounds (Coombe and Iland, 2004; Lund and Bohlmann, 2006; Conde et al., 2007), and affect the synthesis/degradation of certain compounds as polyphenols and anthocyanins (Bergqvist et al., 2001; Spayd et al., 2002; Mori et al., 2007; Teixeira et al., 2013; Zarrouk et al., 2016). In the Campania region, a shifting of the suitable thermal areas for the Aglianico grapevine is expected (Bonfante et al., 2018), resulting in inadequate growing season temperatures, and then immature berries for winemaking. For this reason the potential beneficial effect of fitostimulants on nutritional use efficiency, yield and berry quality traits has recently gained raising interest in grapevine cultivation, despite the limitations related to the yearly different climatic conditions in open-field cultivation systems (Basile et al., 2020 and literature therein). In our study case, the plant extract-induced increase in phenolics' content, coupled with the decrease in soluble sugars in berries, would help counteracting the decoupling between the technological and phenolic maturity.

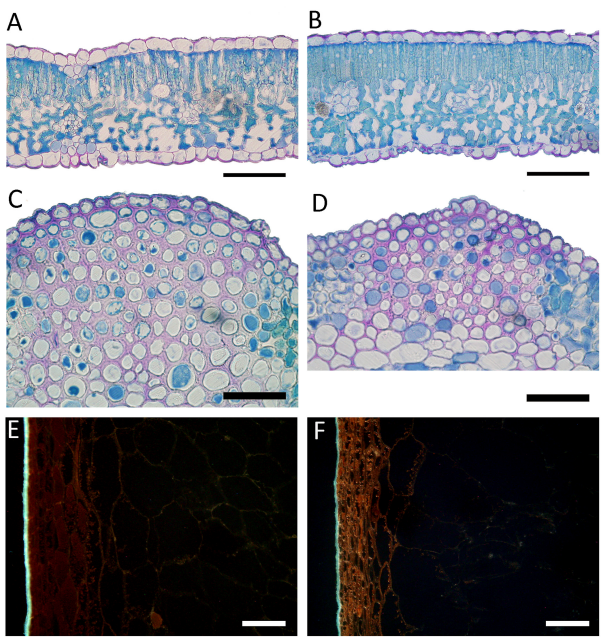

FIGURE 5 | Light **(A–D)** and epi-fluorescence **(E,F)** microscopy views of leaf **(A–D)** and fruit **(E,F)** sections in *V. vinifera* "Aglianico" vines. **(A,B)** leaf cross section of T1 and T3; **(C,D)** detail of the collenchyma in T1 and T3; **(E,F)** fruit longitudinal section showing yellow-orange autofluorescence of phenolics in the sub-epidermal layers in T1 and T3, respectively. Bars = 100 μm in **(A–D)**, 50 μm in **(E,F)**. [T1 – only industry water mixture of copper oxychloride; T3 – half dose of copper oxychloride water mixture].

TABLE 5 | Main effects of year and foliar treatments on leaf mineral composition of *V. vinifera* "Aglianico" vines. Mean values and significance of interactions are shown. Different letters within column indicate significant differences according to Duncan's multiple-range test ($P \leq 0.05$).

	Na^+ (g kg^{-1} DW)	NH_4^+-N (g kg^{-1} DW)	K^+ (g kg^{-1} DW)	Mg^{2+} (g kg^{-1} DW)	Ca^{2+} (g kg^{-1} DW)	NO^{3-}-N (g kg^{-1} DW)	SO_4^{2-} (g kg^{-1} DW)	PO_4^{3-} (g kg^{-1} DW)	Cl^- (g kg^{-1} DW)	Malate (g kg^{-1} DW)	Tartrate (g kg^{-1} DW)	Citrate (g kg^{-1} DW)	Isocitrate (g kg^{-1} DW)
Year (Y)													
2017	0.31^a	0.23^a	14.41^a	3.06^a	12.99^a	0.46^a	3.60^a	0.88^b	0.79^b	31.16^b	71.59^a	2.51^a	0.91^a
2018	0.20^b	0.27^a	5.69^b	1.03^b	7.44^b	0.24^b	1.86^b	1.28^a	1.27^a	35.43^a	47.88^b	1.75^b	0.33^b
Foliar treatment (T^1)													
T1	0.25^a	0.29^a	10.33^b	1.93^b	8.95^b	0.53^a	2.91^a	1.06^a	1.33^a	30.50^c	57.82^a	1.91^c	0.55^c
T2	0.28^a	0.20^a	10.05^b	1.73^b	8.42^b	0.35^b	2.68^a	1.10^a	1.02^b	$31.99^{b,c}$	61.62^a	$2.08^{a,b}$	$0.64^{a,b}$
T3	0.26^a	0.21^a	8.03^c	1.83^b	9.17^b	0.24^b	2.70^a	1.14^a	0.94^b	$34.39^{a,b}$	58.91^a	$2.20^{a,b}$	0.71^a
T4	0.23^a	0.31^a	11.80^a	2.68^a	14.31^a	0.28^b	2.63^a	1.02^a	0.81^b	36.31^a	60.61^a	2.31^a	$0.59^{b,c}$
Significance[2]													
Y	*	NS	***	***	***	***	***	***	***	**	***	***	***
T	NS	NS	***	***	***	***	NS	NS	*	*	NS	*	***
Y × T	NS	NS	***	***	***	***	*	NS	**	***	NS	NS	***

[1] T1 – only industry water mixture of copper oxychloride; T2 – industry water mixture of copper oxychloride plus Trym®; T3 – half dose of copper oxychloride water mixture; T4 – half dose of copper oxychloride water mixture plus Trym®.
[2] NS,, **, ***: Non-significant or significant at $P \leq 0.05$, 0.01, 0.005, respectively.*

TABLE 6 | Main effects of year and foliar treatments on fruit mineral composition of *V. vinifera* "Aglianico" vines. Mean values and significance of interactions are shown. Different letters within column indicate significant differences according to Duncan's multiple-range test ($P \leq 0.05$).

	Na^+ (g kg^{-1} DW)	NH_4^+-N (g kg^{-1} DW)	K^+ (g kg^{-1} DW)	Mg^{2+} (g kg^{-1} DW)	Ca^{2+} (g kg^{-1} DW)	NO_3^--N (g kg^{-1} DW)	SO_4^{2-} (g kg^{-1} DW)	PO_4^{3-} (g kg^{-1} DW)	Cl^- (g kg^{-1} DW)	Malate (g kg^{-1} DW)	Tartrate (g^-kg^{-1} DW)
Year (Y)											
2017	0.024^a	0.046^b	1.471^a	0.071^a	0.074^a	0.022^a	0.159^a	0.043^b	0.063^a	0.155^b	9.667^a
2018	0.033^a	0.059^a	1.402^a	0.042^b	0.072^a	0.003^b	0.156^a	0.166^a	0.022^b	1.557^a	5.355^b
Foliar treatment (T^1)											
T1	0.019^b	0.052^b	1.534^a	0.057^a	0.093^a	0.014^a	0.162^a	0.089^b	0.068^a	0.738^a	7.977^a
T2	0.022^b	0.066^a	1.543^a	0.051^a	0.051^a	0.010^a	0.163^a	0.100^b	0.028^a	0.923^a	8.059^a
T3	0.048^a	0.044^b	1.558^a	0.057^a	0.085^a	0.015^a	0.154^a	0.141	0.043^a	0.968^a	7.898^a
T4	0.024^b	0.048^b	1.117^b	0.060^a	0.063^a	0.009^a	0.150^a	0.089^b	0.032^a	0.798^a	6.112^b
Significance[2]											
Y	NS	**	NS	***	NS	**	NS	***	***	***	***
T	*	**	***	NS	NS	NS	NS	**	NS	NS	***
Y × T	NS	NS	**	NS	NS	NS	NS	NS	NS	NS	NS

[1] T1 – only industry water mixture of copper oxychloride; T2 – industry water mixture of copper oxychloride plus Trym®; T3 – half dose of copper oxychloride water mixture; T4 – half dose of copper oxychloride water mixture plus Trym®.
[2] NS,, **, ***: Non-significant or significant at $P \leq 0.05$, 0.01, 0.005, respectively.*

This is in agreement with other studies reporting the distribution of biostimulants to delay the sugar accumulation (technological maturity), while favoring the achievement of phenolic maturity at harvest as a strategy to control sugar and phenolic accumulation during the ripening process (Salvi et al., 2016). Indeed, the use of aminoacid-based biostimulants is a widespread practice also in other crops as a way to improve the content of phenolics like flavonoids with ROS scavenger activity (Kocira, 2019). The increase in phenolics is also influenced by many other factors, especially drought and nutritional stresses: for example, the reduced levels of nitrogen found in the leaves compared to the control treatment would also have enhanced the accumulation of secondary metabolites (Downey et al., 2006; Król et al., 2014). On the contrary, the application of the plant extract did not reduce nitrogen concentration that is important for adequate fermentation (Bisson and Butzke, 2000). The reduction in Cu distribution likely induced the activation of a plant response also linked with the improvement of mechanical defenses at the leaf level, in particular based on the thickening of the collenchyma at the upper side of veins. Such a thickening was also stimulated by the application of the Trym®. The increased thickness of collenchyma layers and/or the thickening of the cell walls has been reported in grapevine as a leaf response to stress and is considered a strategy to protect the leaves not only from mechanical injuries, but also from fungal invasion (Nicole et al., 1992; Rhimi et al., 2016). The thickening of subepidermal

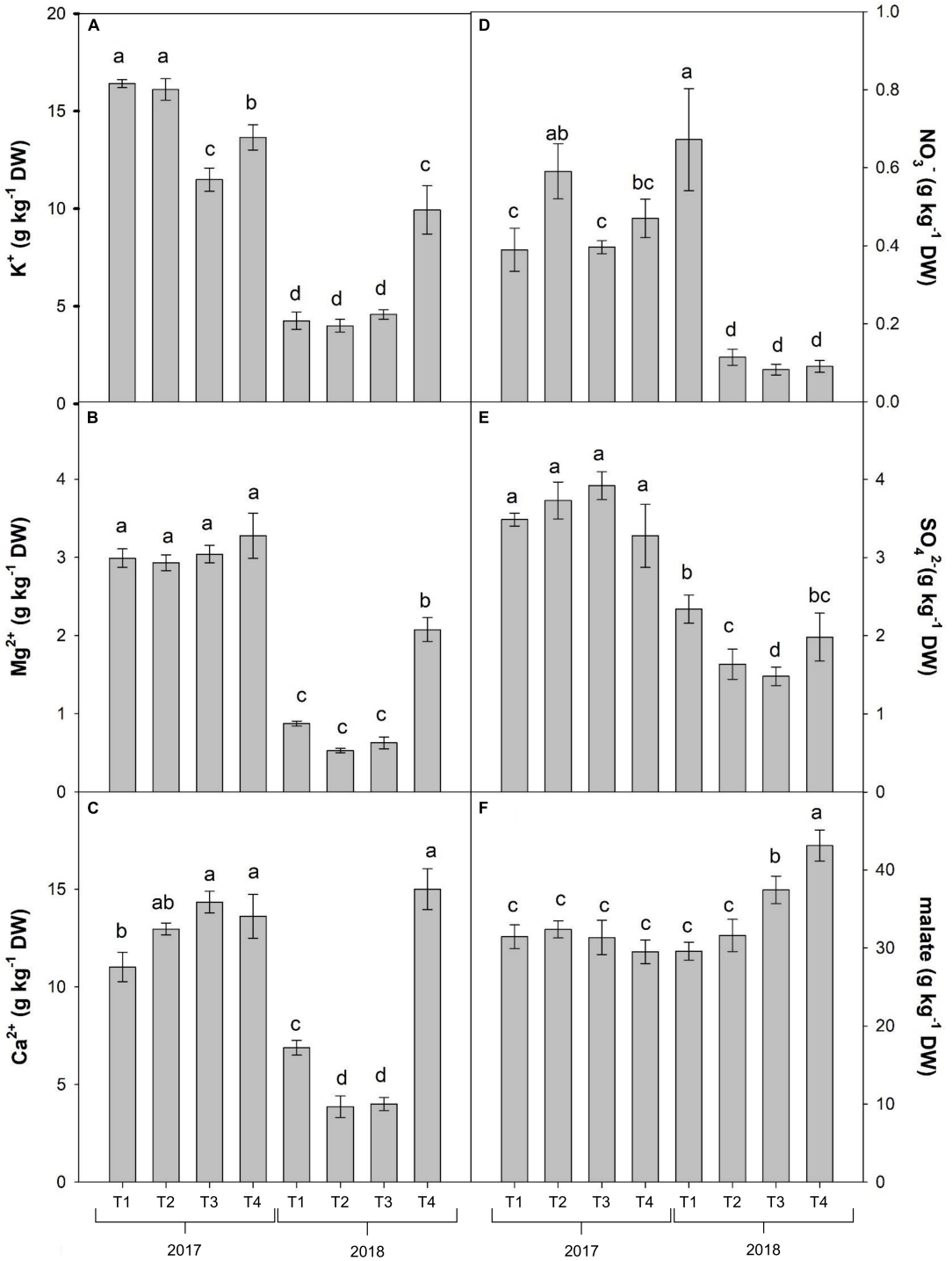

FIGURE 6 | Combined effects of year and foliar treatments on leaf content of potassium **(A)**, magnesium **(B)**, calcium **(C)**, nitrate **(D)**, sulfate **(E)**, and malate **(F)** of *V. vinifera* "Aglianico" vines (mean ± SE). Different letters indicate significant differences according to Duncan's multiple-range test ($P \leq 0.05$). [T1 – only industry water mixture of copper oxychloride; T2 – industry water mixture of copper oxychloride plus Trym®; T3 – half dose of copper oxychloride water mixture; T4 – half dose of copper oxychloride water mixture plus Trym®].

layers of fruits also goes in this direction: subepidermal layers of cells of berries are characterized by elongated cells, parallel to the skin, and whose size and cell wall thickness are respectively much smaller and thicker compared to the cells of the inner layers of the flesh. Therefore, a thicker layer of subepidermal cells indicates a higher resistance of fruits to mechanical injury

and pathogen attack (De Micco and Aronne, 2012). In the vines treated with half Cu dose plus Trym®, the occurrence of phenolics at the periphery of berries would also protect them from excessive solar radiation, which could be consequent to the reduced total leaf area, thus limiting radiation-induced oxidative damage by protecting the membranes (Lattanzio et al., 2008).

In conclusion, the foliar application of the tropical plant extract induced a differential response depending on the environmental factors and on the oxycloride copper dose distributed, with promising implications on Aglianico vegetative growth regulation, on improvement in leaf mechanical and berry antioxidant defenses, as well as on berry quality traits.

The applied multidisciplinary approach proved to be useful to achieve a comprehensive understanding of the vine behavior in the continuum soil-plant-atmosphere, thus providing information as valuable inputs to manage terroirs in the sight of climate change and chemical control restrictions.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial contribution to the work. CC, YR, AE, and VDM designed the study. CC, RC, ChA, FP, EV, AE, AB, and VDM contributed to fieldwork. CC, CA, RC, SDF, EV, AE, and VDM contributed to the laboratory analyses. CC, FP, SDF, and VDM performed statistical analyses. CC, CA, YR, AB, and VDM contributed to the interpretation of results. CC, CA, YR, and VDM wrote the main part of the manuscript. All authors contributed to specific parts of the text, supervised the final draft, and approved the final version of the manuscript.

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REFERENCES

Basile, B., Rouphael, Y., Colla, G., Soppelsa, S., and Andreotti, C. (2020). Appraisal of emerging crop management opportunities in fruit trees, grapevines and berry crops facilitated by the application of biostimulants. *Sci. Hortic.* 267:109330. doi: 10.1016/j.scienta.2020.109330

Bavaresco, L., Civardi, S., Pezzutto, S., Vezzulli, S., and Ferrari, F. (2005). Grape production, technological parameters, and stilbenic compounds as affected by lime-induced chlorosis. *Vitis* 44, 63–65.

Bergqvist, J., Dokoozlian, N., and Ebisuda, N. (2001). Sunlight exposure and temperature effects on berry growth and composition of cabernet sauvignon and grenache in the Central San Joaquin Valley of California. *Am. J. Enol. Vitic.* 52, 1–7. doi: 10.1130/spe234-p1

Bilger, W., and Björkman, O. (1990). Role of the xanthophyll cycle in photoprotection elucidated by measurements of light-induced absorbance changes, fluorescence and photosynthesis in leaves of Hedera canariensis. *Photosyn. Res.* 25, 173–185. doi: 10.1007/BF00033159

Bisson, L. F., and Butzke, C. E. (2000). Diagnosis and rectification of stuck and sluggish fermentations. *Am. J. Enol. Vitic.* 51, 168–177.

Bonfante, A., Monaco, E., Langella, G., Mercogliano, P., Bucchignani, E., Manna, P., et al. (2018). A dynamic viticultural zoning to explore the resilience of terroir concept under climate change. *Sci. Total Environ.* 624, 294–308. doi: 10.1016/j.scitotenv.2017.12.035

Bondada, B. K., and Keller, M. (2012). Not all shrivels are created equal—Morpho-anatomical and compositional characteristics differ among different shrivel types that develop during ripening of grape (*Vitis vinifera L.*) berries. *Am. J. Plant Sci.* 3, 879–898. doi: 10.4236/ajps.2012.37105

Brook, A., De Micco, V., Battipaglia, G., Erbaggio, A., Ludeno, G., Catapano, I., et al. (2020). A smart multiple spatial and temporal resolution system to support precision agriculture from satellite images: proof of concept on Aglianico vineyard. *Remote Sens. Environ.* 240:111679. doi: 10.1016/j.rse.2020.111679

Caccavello, G., Giaccone, M., Scognamiglio, P., Forlani, M., and Basile, B. (2017). Influence of intensity of post-veraison defoliation or shoot trimming on vine physiology, yield components, berry and wine composition in *Aglianico grapevines*. *Aust. J. Grape Wine Res.* 23, 226–239. doi: 10.1111/ajgw.12263

Calvo, P., Nelson, L., and Kloepper, J. W. (2014). Agricultural uses of plant biostimulants. *Plant Soil* 383, 3–41. doi: 10.1007/s11104-014-2131-8

Caradonia, F., Battaglia, V., Righi, L., Pascali, G., and La Torre, A. (2019). Plant biostimulant regulatory framework: prospects in europe and current situation at international level. *J. Plant Growth Regul.* 38, 438–448. doi: 10.1007/s00344-018-9853-4

Carillo, P., Cirillo, C., De Micco, V., Arena, C., De Pascale, S., and Rouphael Y. (2019). Morpho-anatomical, physiological and biochemical adaptive responses to saline water of *Bougainvillea spectabilis* Willd. trained to different canopy shapes. *Agric. Water Manag.* 212, 12–22. doi: 10.1016/j.agwat.2018.08.037

Chaves, M. M., Santos, T. P., Souza, C. D., Ortuño, M. F., Rodrigues, M. L., Lopes, C. M., et al. (2007). Deficit irrigation in grapevine improves water-use efficiency while controlling vigour and production quality. *Ann. Appl. Biol.* 150, 237–252. doi: 10.1111/j.1744-7348.2006.00123.x

Chaves, M. M., Zarrouk, O., Francisco, R., Costa, J. M., Santos, T., Regalado, T., et al. (2010). Grapevine under deficit irrigation: hints from physiological and molecular data. *Ann. Bot.* 105, 661–676. doi: 10.1093/aob/mcq030

Chuang, P. H., Lee, C. W., Chou, J. Y., Murugan, M., Shieh, B. J., and Chen, H. M. (2007). Anti-fungal activity of cruder extractsand essential oil of *Moringa oleifera* Lam. *Bioresour. Technol.* 98, 232–236. doi: 10.1016/j.biortech.2005.11.003

Cirillo, C., De Micco, V., Arena, C., Pannico, A., De Pascale, S., and Rouphael Y. (2019). Biochemical, Physiological and Anatomical Mechanisms of Adaptation of *Callistemon citrinus* and *Viburnum lucidum* to NaCl and $CaCl_2$ Salinization. *Front. Plant Sci.* 10:742. doi: 10.3389/fpls.2019.00742

Cohen, Y., Wang, W. Q., Ben-Daniel, B. H., and Ben-Daniel, Y. (2006). Extracts of *Inula viscosa* control downy mildew of grapes caused by *Plasmopara viticola*. *Phytopathology* 96, 417–424. doi: 10.1094/phyto-96-0417

Colla, G., Hoagland, L., Ruzzi, M., Cardarelli, M., Bonini, P., Canaguier, R., et al. (2017). Biostimulant action of protein hydrolysates: unraveling their effects on plant physiology and microbiome. *Front. Plant Sci.* 8:2202. doi: 10.3389/fpls.2017.02202

Colla, G., Nardi, S., Cardarelli, M., Ertani, A., Lucini, L., Canaguier, R., et al. (2015). Protein hydrolysates as biostimulants in horticulture. *Sci. Hortic.* 196, 28–38. doi: 10.1016/j.scienta.2015.08.037

Conde, C., Silva, P., Fontes, N., Dias, A. C. P., Tavares, R. M., Sousa, M. J., et al. (2007). Biochemical changes throughout grape berry development and fruit and wine quality. *Food* 1, 1–22. doi: 10.1111/j.1755-0238.1996.tb00087.x

Coombe, B., and Iland, P. (2004). "Grape berry development and winegrape quality," in *Viticulture*, Vol. 1, eds P. R. Dry and B. G. Coombe (Adelaide: Winetitles), 210–248.

Cornelissen, J. H. C., Lavorel, S., Garnier, E., Díaz, S., Buchmann, N., Gurvich, D. E., et al. (2003). A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Aust. J. Bot.* 51, 335–380. doi: 10.1071/BT02124

Costa, M. H., Yanagi, S. N., Souza, P. J., Ribeiro, A., and Rocha, E. J. (2007). Climate change in Amazonia caused by soybean cropland expansion, as compared to caused by pastureland expansion. *Geophys. Res. Lett.* 34:L07706. doi: 10.1029/2007GL029271

Dagostin, S., Schärer, H.-J., Pertot, I., and Tamm, L. (2011). Are there alternative to copper for controlling grapevine downy mildew in organic viticulture? *Crop Prot.* 30, 776–788. doi: 10.1016/j.cropro.2011.02.031

De Micco, V., and Aronne, G. (2007). Combined histochemistry and autofluorescence for identifying lignin distribution in cell walls. *Biotech. Histochem.* 82, 209–216. doi: 10.1080/10520290701713981

De Micco, V., and Aronne, G. (2012). "Morpho-anatomical traits for plant adaptation to drought," in *Plant Responses to Drought Stress*, ed. R. Aroca (Berlin: Springer), 37–61. doi: 10.1007/978-3-642-32653-0_2

Downey, M. O., Dokoozlian, N. K., and Krstic, M. (2006). Cultural practice and environmental impacts on the flavonoid composition of grapes and wine: a review of recent research. *Am. J. Enol. Vitic.* 3, 257–268.

du Jardin, P. (2012). *The Science of Plant Biostimulants–A Bibliographic Analysis, Ad Hoc Study Report*. Brussels: European Commission.

du Jardin, P. (2015). Plant biostimulants: definition, concept, main categories and regulation. *Sci. Hortic.* 196, 3–14. doi: 10.1016/j.scienta.2015.09.021

Ertani, A., Francioso, O., Tugnoli, V., Righi, V., and Nardi, S. (2011). Effect of commercial lignosulfonate-humate on *Zea mays* L. metabolism. *J. Agric. Food Chem.* 59, 11940–11948. doi: 10.1021/jf202473e

European Union [EU] (1990). *Commission Regulation (EEC) No 2676/90 (REGULATION, H. A. T. 1990. Commission Regulation (EEC) No. 2676/90 Determining Community Methods for the Analysis of Wines Official Journal L 272, 3 October 1990*. Brussels: European Union, 1–192.

European Union [EU] (2019). *REGULATION OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL laying down rules on the making available on the market of EU fertilising products and amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009 and repealing Regulation (EC) No 2003/2003*. Brussels: European Union.

FAOSTAT (2020). Available online at: http://www.fao.org/faostat (accessed June 20, 2020).

Feder, N. E. D., and O'Brien, T. P. (1968). Plant microtechnique: some principles and new methods. *Am. J. Bot.* 55, 123–142. doi: 10.2307/2440500

Ferrara, G., and Brunetti, G. (2010). Effects of the times of application of a soil humic acid on berry quality of table grape (*Vitis vinifera* L.) cv Italia. *Span J. Agric. Res.* 8, 1–6. doi: 10.5424/1283

Franceschi, V. R., Krekling, T., Berryman, A. A., and Christiansen, E. (1998). Specialized phloem parenchyma cells in Norway spruce (Pinaceae) bark are an important site of defense reactions. *Am. J. Bot.* 85, 601–615. doi: 10.2307/2446529

Frioni, T., Sabbatini, P., Tombesi, S., Norrie, J., Poni, S., Gatti, M., et al. (2018). Effects of a biostimulant derived from the brown seaweed Ascophyllum nodosum on ripening dynamics and fruit quality of grapevines. *Sci. Hortic.* 232, 97–106. doi: 10.1016/j.scienta.2017.12.054

Fukazawa, K. (1992). *Ultraviolet Microscopy. In Methods in Lignin Chemistry*. Berlin: Springer, 110–121.

Genty, B., Briantais, J. M., and Baker, N. R. (1989). The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *BBA Gen. Subjects* 990, 87–92. doi: 10.1016/S0304-4165(89)80016-9

Goode, J. (2012). Viticulture: fruity with a hint of drought. *Nature* 492, 351–352. doi: 10.1038/492351a

Graham, L. E., Kodner, R. B., Fisher, M. M., Graham, J. M., Wilcox, L. W., Hackney, J. M., et al. (2004). "Early land plant adaptations to terrestrial stress: a focus on phenolics," in *The Evolution of Plant Physiology*, eds A. R. Hemsley and I. Poole (Boston, MA: Elsevier Academic Press), 155–169. doi: 10.1016/b978-012339552-8/50010-x

Gransee, A., and Führs, H. (2013). Magnesium mobility in soils as a challenge for soil and plant analysis, magnesium fertilization and root uptake under adverse growth conditions. *Plant Soil* 368, 5–21. doi: 10.1007/s11104-012-1567-y

Gutiérrez-Gamboa, G., Romanazzi, G., Garde-Cerdán, T., and Pérez-Álvarez, E. P. (2019). A review of the use of biostimulants in the vineyard for improved grape and wine quality: effects on prevention of grapevine diseases. *J. Sci. Food Agric.* 99, 1001–1009. doi: 10.1002/jsfa.9353

IPCC (2014). "2014: climate change 2014: synthesis report," in *Proceedings of the Contribution of Working Groups I. II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*, (Geneva: IPCC).

IUSS Working Group WRB (2015). *International Soil Classification System for Naming Soils and Creating Legends for Soil Maps. World Reference Base for Soil Resources 2014, Update 2015, 106*. Greenwich: IUSS Working Group WRB.

Jones, G. V., and Webb, L. B. (2010). Climate change, viticulture, and wine: challenges and opportunities. *Int. J. Wine Res.* 21, 103–106. doi: 10.1080/09571264.2010.530091

Kocira, S. (2019). Effect of amino acid biostimulant on the yield and nutraceutical potential of soybean. *Chil. J. Agric. Res.* 79, 17–25. doi: 10.4067/S0718-58392019000100017

Król, A., Amarowicz, R., and Weidner, S. (2014). Changes in the composition of phenolic compounds and antioxidant properties of grapevine roots and leaves (*Vitis vinifera* L.) under continuous of long-term drought stress. *Acta Physiol. Plant.* 36, 1491–1499. doi: 10.1007/s11738-014-1526-8

Kumar, P., Lucini, L., Rouphael, Y., Cardarelli, M., Kalunke, R. M., and Colla, G. (2015). Insight into the role of grafting and arbuscular mycorrhiza on cadmium stress tolerance in tomato. *Front. Plant Sci.* 6:477. doi: 10.3389/fpls.2015.00477

Lattanzio, V., Kroon, P. A., Quideau, S., and Treutter, D. (2008). Plant phenolics—secondary metabolites with diverse functions. *Recent Adv. Polyphenol. Res.* 1, 1–35. doi: 10.1002/9781444302400.ch1

Lereboullet, A. L., Bardsley, D., and Beltrando, G. (2013). Assessing vulnerability and framing adaptive options of two Mediterranean wine growing regions facing climate change: roussillon (France) and McLaren Vale (Australia). *EchoGéo* 23:13384. doi: 10.4000/echogeo.13384

Lereboullet, A. L., Beltrando, G., Bardsley, D. K., and Rouvellac, E. (2014). The viticultural system and climate change: coping with long-term trends in temperature and rainfall in Roussillon. *France. Reg. Environ. Change* 14, 1951–1966. doi: 10.1007/s10113-013-0446-2

Lichtenthaler, H. K. (1987). Chlorophylls and carotenoids, the pigments of photosynthetic biomembranes. *Methods Enzymol.* 148, 350–382. doi: 10.1016/0076-6879(87)48036-1

Lopes, L. D., Böger, B. R., Cavalli, K. F., Silveira-Júnior, J. F. D. S., Osório, D. V., de Oliveira, S., et al. (2014). Fatty acid profile, quality lipid index and bioactive compounds of flour from grape residues. *Agric. Nat. Resour.* 41, 225–234. doi: 10.4067/S0718-16202014000200009

Lorenz, D. H., Eichhorn, K. W., Bleiholder, H., Klose, R., Meier, U., and Weber, E. (1995). Growth stages of the grapevine: phenological growth stages of the grapevine (*Vitis vinifera* L. ssp. vinifera)—Codes and descriptions according to the extended BBCH scale. *Aust. J. Grape Wine Res.* 1, 100–103. doi: 10.1111/j.1755-0238.1995.tb00085.x

Lund, S. T., and Bohlmann, J. (2006). The molecular basis for wine grape quality-a volatile subject. *Science* 311, 804–805. doi: 10.1126/science.1118962

Mancuso, S., Azzarello, E., Mugnai, S., and Briand, X. (2006). Marine bioactive substances (IPA extract) improve foliar ion uptake and water stress tolerance in potted Vitis vinifera plants. *Adv. Hortic. Sci.* 20, 156–161. doi: 10.1400/53262

Martínez-Gil, A. M., Angenieux, M., Pardo-García, A. I., Alonso, G. L., Ojeda, H., and Salinas, M. R. (2013). Glycosidic aroma precursors of Syrah and Chardonnay grapes after an oak extract application to the grapevines. *Food Chem.* 138, 956–965. doi: 10.1016/j.foodchem.2012.11.032

Martínez-Gil, A. M., Garde-Cerdán, T., Zalacain, A., Pardo-García, A. I., and Salinas, M. R. (2012). Applications of an oak extract on Petit Verdot grapevines. Influence on grape and wine volatile compounds. *Food Chem.* 132, 1836–1845. doi: 10.1016/j.foodchem.2011.12.016

Medrano, H., Escalona, J. M., Cifre, J., Bota, J., and Flexas, J. (2003). A ten-year study on the physiology of two Spanish grapevine cultivars under field

conditions: effects of water availability from leaf photosynthesis to grape yield and quality. *Funct. Plant Biol.* 30, 607–619. doi: 10.1071/FP02110

Mori, K., Goto-Yamamoto, N., Kitayama, M., and Hashizume, K. (2007). Loss of anthocyanins in red-wine grape under high temperature. *J. Exp. Bot.* 58, 1935–1945. doi: 10.1093/jxb/erm055

Nicole, M. R., Geiger, J. P., and Nandris, D. (1992). "Defense of angiosperm roots against fungal invasion," in *Defense Mechanisms of Woody Plants Against Fungi*, eds R. A. Blanchette and A. R. Biggs (Berlin: Springer), 181–206. doi: 10.1007/978-3-662-01642-8_10

Palliotti, A., Tombesi, S., Silvestroni, O., Lanari, V., Gatti, M., and Poni, S. (2014). Changes in vineyard establishment and canopy management urged by earlier climate-related grape ripening: a review. *Sci. Hortic.* 178, 43–54. doi: 10.1016/j.scienta.2014.07.039

Parrado, J., Escudero-Gilete, M. L., Friaza, V., García-Martínez, A., González-Miret, M. L., Bautista, J. D., et al. (2007). Enzymatic vegetable extract with bio-active components: influence of fertiliser on the colour and anthocyanins of red grapes. *J. Sci. Food Agric.* 87, 2310–2318. doi: 10.1002/jsfa.2989

Poni, S., Gatti, M., Palliotti, A., Dai, Z., Duchêne, E., Truong, T. T., et al. (2018). Grapevine quality: a multiple choice issue. *Sci. Hortic.* 234, 445–462. doi: 10.1016/j.scienta.2017.12.035

Popescu, G. C., and Popescu, M. (2018). Yield, berry quality and physiological response of grapevine to foliar humic acid application. *Bragantia* 77, 273–282. doi: 10.1590/1678-4499.2017030

Rhimi, N., Ben Ahmed, C., Elloumi, N., Athar, H. R., Noreen, S., Ashraf, M., et al. (2016). Morpho-anatomical and physiological changes in grapevine leaves exposed to amospheric fluoride and sulfur dioxide pollution. *Appl. Ecol. Environ. Res.* 14, 77–89. doi: 10.15666/aeer/1405_077089

Rogiers, S. Y., and Clarke, S. J. (2013). Nocturnal and daytime stomatal conductance respond to root-zone temperature in 'Shiraz' grapevines. *Ann. Bot.* 111, 433–444. doi: 10.1093/aob/mcs298

Rouphael, Y., and Colla, G. (2018). Synergistic biostimulatory action: designing the next generation of plant biostimulants for sustainable agriculture. *Front. Plant Sci.* 9:1655. doi: 10.3389/fpls.2018.01655

Rouphael, Y., and Colla, G. (2020). Biostimulants in agriculture. *Front. Plant Sci.* 11:40. doi: 10.3389/fpls.2020.00040

Rusjan, D., Strlič, M., Pucko, D., and Korošec-Koruza, Z. (2007). Copper accumulation regarding the soil characteristics in Sub-Mediterranean vineyards of Slovenia. *Geoderma* 141, 111–118. doi: 10.1016/j.geoderma.2007.05.007

Ruzin, S. E. (1999). *Plant Microtechnique and Microscopy*, Vol. 198. New York, NY: Oxford University Press.

Salvi, L., Cataldo, E., Secco, S., and Mattii, G. B. (2016). Use of natural biostimulants to improve the quality of grapevine production: first results. *Acta Hortic.* 1148, 77–84. doi: 10.17660/ActaHortic.2016.1148.9

Sánchez-Gómez, R., Garde-Cerdán, T., Zalacain, A., Garcia, R., and Cabrita, M. J. (2016a). Vine-shoot waste aqueous extract applied as foliar fertilizer to grapevines: effect on amino acids and fermentative volatile content. *Food Chem.* 197, 132–140. doi: 10.1016/j.foodchem.2015.10.034

Sánchez-Gómez, R., Zalacain, A., Pardo, F., Alonso, G. L., and Salinas, M. R. (2016b). An innovative use of vine-shoots residues and their "feedback" effect on wine quality. *Innov. Food Sci. Emerg. Technol.* 37, 18–26. doi: 10.1016/j.ifset.2016.07.021

Schiavon, M., Pizzeghello, D., Muscolo, A., Vaccaro, S., Francioso, O., and Nardi, S. (2010). High molecular size humic substances enhance phenylpropanoid metabolism in maize (*Zea mays* L.). *J. Chem. Ecol.* 36, 662–669. doi: 10.1007/s10886-010-9790-6

Spayd, S. E., Tarara, J. M., Mee, D. L., and Ferguson, J. C. (2002). Separation of sunlight and temperature effects on the composition of *Vitis vinifera* cv. Merlot berries. *Am. J. Enol. Vitic.* 53, 171–182.

Teixeira, A., Eiras-Dias, J., Castellarin, S. D., and Gerós, H. (2013). Berry phenolics of grapevine under challenging environments. *Int. J. Mol. Sci.* 14, 18711–18739. doi: 10.3390/ijms140918711

Valverde, P., de Carvalho, M., Serralheiro, R., Maia, R., Ramos, V., and Oliveira, B. (2015). Climate change impacts on rainfed agriculture in the Guadiana river basin (Portugal). *Agric. Water Manag.* 150, 35–45. doi: 10.1016/j.agwat.2014.11.008

Vogt, T. (2010). Phenylpropanoid biosynthesis. *Mol. Plant* 3, 2–20. doi: 10.1093/mp/ssp106

Webb, L. B., Watterson, I., Bhend, J., Whetton, P. H., and Barlow, E. W. R. (2013). Global climate analogues for winegrowing regions in future periods: projections of temperature and precipitation. *Aust. J. Grape Wine Res.* 19, 331–341. doi: 10.1111/ajgw.12045

Yakhin, O. I., Lubyanov, A. A., Yakhin, I. A., and Brown, P. H. (2017). Biostimulants in plant science: a global perspective. *Front. Plant Sci.* 7:2049. doi: 10.3389/fpls.2016.02049

Zarrouk, O., Brunetti, C., Egipto, R., Pinheiro, C., Genebra, T., Gori, A., et al. (2016). Grape ripening is regulated by deficit irrigation/elevated temperatures according to cluster position in the canopy. *Front. Plant Sci.* 7:1640. doi: 10.3389/fpls.2016.01640

Zhifeng, G., and Chengguang, F. (1994). Determination of organic acids and inorganic anions by single-column ion chromatography. *J. Instrum. Anal.* 5, 32–36.

Zhu, Q., Zhang, M., and Ma, Q. (2012). Copper-based foliar fertilizer and controlled release urea improved soil chemical properties, plant growth and yield of tomato. *Sci. Hortic.* 143, 109–114. doi: 10.1016/j.scienta.2012.06.008

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