UNEXPECTED RELATIONSHIPS BETWEEN $\delta^{13}C$ AND WINE GRAPE PERFORMANCE IN ORGANIC FARMING

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Abstract

Aim: To evaluate the relationship between carbon isotope ratio ($\delta^{13}C$) and wine grape viticultural and oenological performance in organic farming.

Methods and results: The study was carried out for four years in the Chianti Classico wine production district (Central Italy), on five non irrigated vineyards conducted in organic farming. The reference variety was Sangiovese. Eleven sites were chosen for vine monitoring and grape sampling. The performance parameters were alcohol and must sugar content, sugar accumulation rate, mean berry weight, and extractable polyphenols. $\delta^{13}C$, stem water potential, and soil water availability were also monitored. Finally, soil nitrogen as well as yeast available nitrogen in the must were measured. $\delta^{13}C$ was directly related to stem water potential and soil water deficit, and indicated a range of water stress conditions from none and moderate to strong. However, its relationship with viticultural and oenological results was contrary to expectation, that is, performance linearly increased along with soil moisture. On the other hand, the worst performance was obtained where both water and nitrogen were more limiting.

Conclusions: The unexpected relationship between $\delta^{13}C$ and Sangiovese performance was caused by low nitrogen availability. The studied sites all had low-fertility soils with poor or very poor nitrogen content. Therefore, in the plots where soil humidity was relatively higher, nitrogen plant uptake was favoured, and Sangiovese performance improved. Macronutrient being the main limiting factor, the performance was not lower in the plots where soil water availability was relatively larger. Therefore, the best viticultural result was obtained with no water stress conditions, at low rather than at intermediate $\delta^{13}C$ values.

Significance and impact of the study: Water nutrition is crucial for wine grape performance. $\delta^{13}C$ is a method used to assess vine water status during the growing season and to estimate vine performance. A good performance is expected at moderate stress and intermediate $\delta^{13}C$ values. A better knowledge of the interaction between water and nutrient scarcity is needed, as it can affect the use of $\delta^{13}C$ to predict vine performance.

Key words: carbon isotopes, water availability, nitrogen, red grape, performance index, Tuscany

Résumé

Méthodes et résultats: L'essai a été réalisé pendant quatre ans dans la région du Chianti Classico (Italie centrale), sur vignes non irriguées conduites en agriculture biologique. La variété de référence est le Sangiovese. Onze sites ont été choisis pour le suivi de la vigne et la maturation du raisin. Les variables mesurées pour évaluer la performance ont été l'alcool et la teneur en sucre dans le moût, le taux d'accumulation de sucre, le poids moyen des baies, et les polyphénols extractibles. Le $\delta^{13}C$, le potentiel hydrique de tige, et la disponibilité en eau du sol ont été suivis. L'azote du sol ainsi que l'azote assimilable dans le moût ont été mesurés. Le $\delta^{13}C$ était corrélé avec le potentiel hydrique de tige et le déficit en eau du sol, et a indiqué une gamme de stress hydrique entre absent et modéré à fort. Cependant, la relation avec les résultats viticoles et œnologiques était contraire à celle attendue. L'indice de performance a augmenté linéairement lorsque le stress hydrique a diminué. D'autre part, la moins bonne performance a été obtenue dans les situations où l'eau et l'azote ont été limitants.

Conclusions: La relation inattendue entre $\delta^{13}C$ et la performance du Sangiovese a été provoquée par la faible disponibilité en azote. Les sols étudiés ont tous été d'une faible fertilité et d'une teneur en azote faible à très faible. Par conséquent, dans les parcelles où l'humidité du sol était relativement plus élevée, l'absorption de l'azote par la plante a été favorisée, ce qui a amélioré la performance du Sangiovese. Dans des conditions où l'azote a été le principal facteur limitant, la performance n'a jamais été négativement affectée par la disponibilité en eau du sol. Par conséquent, le meilleur résultat a été obtenu dans des conditions sans contrainte hydrique, indiquées par des valeurs de $\delta^{13}C$ fortement négatives.

Signification et impact de l'étude: L'alimentation en eau est cruciale pour la performance de la vigne. $\delta^{13}C$ est une méthode utilisée pour évaluer le degré de contrainte hydrique subi au cours de la saison viticole et d'évaluer la performance de la vigne. Une bonne performance est attendue pour une contrainte hydrique modérée et des valeurs intermédiaires de $\delta^{13}C$. Peu de connaissances existent au sujet de l'interaction entre l'eau et la carence en nutriments. Ces relations peuvent affecter l'utilisation du $\delta^{13}C$ pour prédire le comportement de la vigne.

Mots clés: isotopes du carbone, disponibilité en eau, azote, raisin rouge, performance, Toscane

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INTRODUCTION

The best terroirs for red wine production are those in which some soil factors are limiting to vine vigour and berry size (van Leeuwen and Seguin, 2006), so that grapes ripen completely but slowly. These limiting factors may be chemical (limited nitrogen nutrition or ion antagonisms) or physical (insufficient water supply during certain phases of the growth cycle of the vine).

As a whole, nitrogen nutrition and water supply during certain phases of the growth cycle of the vine are considered essential factors of wine quality. Their role in determining the terroir effect has been experienced in many wine producing areas and with several varieties including, among others, France, with Cabernet-Sauvignon (Choné *et al.*, 2001), Merlot (Tregoat *et al.*, 2002) and Sauvignon Blanc (Peyrot des Gachons *et al.*, 2005), Australia with Sauvignon Blanc (White *et al.*, 2007), Hungary with Kékfrankos (Zsófi *et al.*, 2009), and USA with Cabernet-Sauvignon and Chardonnay (Chapman *et al.*, 2005 ; Deluc *et al.*, 2009).

Grapevine nitrogen nutrition, in particular, influences quality components in the grape and, ultimately, the wine. In addition, fermentation kinetics and formation of flavour-active metabolites are also affected by the nitrogen status of the must (Bell and Henschke, 2005). Plant uptake of soil nitrogen increases the concentration of major nitrogenous compounds such as total nitrogen, total amino acids, arginine, proline, ammonium and, consequently, yeast-assimilable nitrogen (YAN) in fruit. Intermediate must YAN offers the optimum balance between desirable and undesirable chemical and sensory wine attributes (Bell and Henschke, 2005). When the nitrogen concentration in must is low, there is a direct relationship between initial nitrogen concentration and the total concentration of higher alcohols in the wine (Äyräpää, 1971).

Water supply during specific phases of the growth cycle of the vine is also considered an essential factor of wine quality. A mild water deficit stress, in particular, is considered to enhance grape quality for the production of red wines (van Leeuwen *et al.*, 2009). More specifically, Tregoat *et al.* (2002) found that Merlot development and berry composition were more influenced by water uptake conditions than by vine nitrogen status in Bordeaux. Besides, at similar water uptake conditions, low vine nitrogen status increased berry potential. On the other hand, a moderate compared to a weak water stress increased YAN in grapes of Merlot, Syrah, Viognier, and Chardonnay (Ojeda and Salmon, 2011). Such results, in addition to other more ecological motivations, enhanced the limitation of nitrogen fertilization in premium viticulture and the adoption of biological farming. Currently, organic viticulture covers about 80,000 ha in Europe and 44,000 in Italy, but not much is known on its effective feedback on wine grape performance.

Although knowledge of water and nitrogen nutrition in a vineyard is essential, its assessment is difficult, since soil water and nitrogen content have a strong temporal and 3D spatial variability, especially in rainfed cultivation and biological farming, where the use of synthetic nitrogen fertilizers is not allowed Then, a common practice is the monitoring of viticultural and oenological indicators, like yield components, leaf and stem water potential (Ψstem), leaf analysis, oenological parameters, and sensory assessment.

Recently, in addition to traditional techniques, a new physiologic marker has been introduced for an integrative evaluation of the overall vine water uptake conditions during the ripening period, i. e., the ratio between the two stable carbon isotopes $^{13}C/^{12}C$, called $\delta^{13}C$. Actually, stomata closure under water deficit restricts water and CO_2 flow ; as ^{12}C is preferentially fixed during the photosynthesis process, when stomata are open, this results in a relative increase in the concentration of the heaviest isotope ^{13}C. The isotopic ratio can be measured either in the must sugars upon harvesting or in the alcohol of the wine produced (van Leeuwen *et al.*, 2001 ; Tregoat *et al.*, 2002 ; van Leeuwen *et al.*, 2003), though there is a shift of $\delta^{13}C$ between ethanol and sugars, due to the loss of ^{13}C-enriched carbon atoms of glucose as CO_2 during fermentation. Rossmann *et al.* (1996) found for 1,631 Italian samples a mean shift of 1.7‰.

Reported $\delta^{13}C$ values in the must sugars range from -21.0 to -28.0‰, with -25.5 or -26.0‰ considered threshold values between water deficit and non-limiting water nutrition (van Leeuwen *et al.*, 2003 ; Deschepper *et al.*, 2006). According to the same authors (van Leeuwen *et al.*, 2003 ; Tregoat *et al.*, 2002), values of about -23‰ in the must correspond to a minimum Ψstem close to -12 bar, a value that matched an optimal correlation with the berry sugar accumulation rate. Being correlated with important components of must quality (e.g., sugar content and titratable acidity), $\delta^{13}C$ can also be used to map terroirs at different scales (van Leeuwen *et al.*, 2001 ; Guix-Hébrard *et al.*, 2007 ; Zufferey and Murisier, 2007 ; Reynard *et al.*, 2011 ; Murisier *et al.*, 2012). A good quality performance is expected at moderate water stress and intermediate $\delta^{13}C$ values.

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In the Chianti area (Central Italy) Costantini *et al.* (2010), using the $\delta^{13}C$ to evaluate land suitability for Sangiovese grapevine, could infer that the threshold between good and bad sensory evaluation of wine matched a $\delta^{13}C$ value of -26.7±1.2‰ in the alcohol, corresponding to a slight water deficit and a Ψstem of -9 bar. This value might be chosen as a first reference for the evaluation of Sangiovese performance.

Another method to evaluate the "performance" of the Sangiovese cultivar, that is, the vegetative and productive behaviour in a specific year and soil, was worked out by Bucelli *et al.* (2010). They used a set of selected viticultural parameters in a multivariate regression to estimate wine sensory evaluation (score) at the panel test. The results allowed to identify statistically different classes of wines and Sangiovese responses to year and environment variations.

Not much is known so far about the influence of the interactions between different forms of abiotic stress on $\delta^{13}C$, like those that might occur in rainfed and organic cultivation between water and nutrient scarcity. This research study was set up to test the relationship between $\delta^{13}C$ and Sangiovese viticultural and oenological performance in organic farming.

MATERIALS AND METHODS

1. Experimental setting, climate and soils

The study was carried out for four years, three years of monitoring (2007-2010) and one of validation (2011), in one of the largest and most important farm of the Chianti Classico wine production district (Tuscany, Central Italy), on 5 non irrigated vineyards, which covered about 40 ha. The fourth year of experimentation was conducted to confirm the unexpected results obtained in the three years of monitoring.

The reference variety was Sangiovese, which is the main variety for red wines in Central Italy (Fregoni, 2006 ; Costantini *et al.*, 2006). Plant density was 5,500-6,600 per ha and the rootstock was 420A (*Vitis berlandieri*×*Vitis riparia*), which is considered to be resistant to drought and active lime. Vineyards were planted in 2000, after slope reshaping by bulldozing and back hoe ploughing down to about 0.8-1.0 m. The vineyard was organically farmed and yields were low. Therefore, chemical fertilization was not implemented and only organic compounds (composted manure) were used. Nevertheless, no evident symptoms of nitrogen deficiency were observed. Training system, canopy management, and number of buds per plant were chosen in order to reduce production. Soil surface was periodically cultivated with mechanical tillage to limit weed growth, interrupt capillarity and reduce evaporation, but only at alternate rows, to limit water runoff and soil erosion. During the years 2009 and 2010, composted manure was distributed at the rate of 1,000 kg ha^{-1}.

A weather station was placed near the selected vineyards. Eleven plots, covering about 100 m^2 each, were selected on the basis of a detailed survey, conducted with both traditional and multispectral image analysis and soil geoelectrical investigation (Andrenelli *et al.*, 2011 ; Martini *et al.*, 2013), to represent soil and plant spatial variability within vineyards. Thus, they should be considered representative of large parts of the farm.

In each plot, parallel soil and vine monitoring, as well as field measurements and grape sampling were carried out. However, as the experimental vineyards and plots were not restricted and were all subjected to conventional agricultural management, some plots and monitoring devices were damaged during the study by the wildlife or farm machinery and had to be discarded. Hence, the validation was conducted on only six plots.

The study area was characterized by a Mediterranean sub-oceanic climate (Costantini *et al.*, 2013) with cool and rainy winters, with minimum monthly average air temperatures close to 0 °C, but hot summers, with a relevant number of days with maximum temperature higher than 30 °C (on the long term, June 8.3 days, July 17.5, August 17.3, and September 2.8). Average long-term annual precipitation was 800 mm, concentrated in autumn and springtime, potential evapotranspiration (ET_0) from April to September was about 850 mm (Hargreaves and Samani, 1982), and mean value of the Winkler index was 1,856 Degree Days.

Soil description followed the standard Italian methods (Costantini, 2007) and classification according to the Word Reference Base for Soil Resources (WRB ; FAO-IUSS-ISRIC, 2006). Six soil typological units (STUs) were identified in the study vineyards (Figure 1) : Nebbiano (NEB), Torricella (TOR), Miniera (MIN), Santa Lucia (SLC), and Leccio 1 and Leccio 2 (LEC1, LEC2). The soil profiles at the eleven sites (named BRO1-2, BRO4-7 and BRO9-13) are described in Tables 1 and 2. Nebbiano is a preserved reddish palaeosol, deep, moderately structured and with loamy or fine loamy texture, developed on ancient fluvial terrace deposits (Plio-Pleistocene period) ; two sites were chosen represented by profiles BRO4 and BRO6. The gravel content is moderate (10-25 %) with fine to medium

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particles. The soil is strongly structured and well drained, with low calcium carbonate ($CaCO_3$) content (< 3%), moderate organic matter (1.2-1.7 %), sub-alkaline pH (7.7-8.4), and moderate available water capacity (AWC) (110-125 mm m^{-1}). The soil was classified Cutanic Luvisol (Hypereutric, Profondic, Clayic) according to WRB (FAO-IUSS-ISRIC, 2006). Torricella is a pale-brown, clay-loamy and strongly gravelly (20-50 %) soil, developed on a tertiary calcareous flysch (sites BRO1 and BRO2). The soil is moderately structured, well drained, with high $CaCO_3$ content (about 25 %), moderate organic matter (1.4-2.3 %), sub-alkaline pH (7.6-8.3), and low to moderate AWC (90-115 mm m^{-1}). It was classified Haplic Cambisol (Calcaric, Skeletic). Miniera is a greyish, clayey soil developed on Pliocene marine

Table 1 – Soil profile descriptions.

Site (BRO)	Horizon	Depth (cm)	Moist colour	Redox mottles	Skeleton (%)	Structure	Consistence	Vine roots ($n \cdot m^{-2}$)		
								coarse	medium	fine
1	Ap	20	2.5Y5/4	absent	30	weak	resistent	0.3	0.7	2.0
	Bw	110	2.5Y5/4	10yr5/6, 1%	30	weak	resistent	0.0	1.7	0.0
	BC	130+	2.5Y5/5	10yr5/6, 2%	55	weak	resistent	0.0	0.0	0.0
2	Ap	28	10YR4/3	2.5y7/3, 1%	35	weak	resistent	0.0	0.3	0.7
	Bw	100	2.5Y5/5	absent	35	weak	resistent	0.0	1.7	0.0
	BC	120+	2.5Y4/5	absent	55	weak	resistent	0.0	0.0	0.0
4	Ap1	35	7.5YR4/4	absent	20	weak	friable	0.0	0.7	1.0
	Ap2/A	85	7.5YR5/8	absent	20	weak	friable	0.7	1.3	1.0
	Ap2/Bw	85	7.5YR4/6	absent	25	weak	friable	0.7	1.3	1.0
	Bwg	130+	7.5YR5/8	10yr7/8, 35%	40	weak	resistent	0.3	0.0	0.0
5	Ap	25	10YR4/6	7.5yr5/8,1%	10	weak	resistent	0.0	0.3	0.7
	Bwg	68	10YR4/6	5yr5/8,10%	3	weak	resistent	1.0	1.0	1.0
	Cg1	100+	5Y7/1	absent	1	weak	resistent	0.0	0.7	0.7
6	Ap1	25	10YR4/6	absent	10	weak	friable	0.0	0.3	0.7
	Ap2/A	110	7.5YR4/4	10yr5/4, 3%	20	weak	friable	1.0	1.0	1.0
	Ap2/Btg	110	7.5YR4/6	10yr5/4, 35%	20	moderate	friable	1.0	1.0	1.0
	Btg	140+	10YR5/8	10yr6/3, 5%	3	moderate	resistent	0.0	0.7	0.7
7	Ap1	20	10YR4/4	7.5yr5/6, 3%	3	weak	friable	0.0	2.0	2.0
	Ap2/A	85	2.5Y3/3	absent	2	weak	friable	1.0	1.0	1.0
	Ap2/Bw	105+	10YR4/3	absent	2	weak	friable	0.0	1.0	0.0
9	Ap	20	10YR4/3	10yr6/4, 1%	3	weak	friable	0.3	0.3	5.0
	Bw	70	10YR5/6	10yr6/3, 1%	1	weak	friable	0.7	1.0	1.3
	2Bt	140+	10YR5/4	absent	1	weak	resistent	0.7	0.3	0.0
10	Ap	60	10YR4/6	absent	10	weak	friable	1.0	1.3	2.0
	2Bt	120+	10YR5/4	absent	1	weak	resistent	0.7	0.7	0.0
11	Ap	40	10YR4/6	absent	4	moderate	friable	0.7	1.0	3.3
	Bw	100	10YR5/6	absent	4	moderate	friable	0.7	1.7	2.0
	BC	130+	10YR6/8	10yr7/2, 1%	15	massive	friable	0.3	2.3	0.3
12	Ap	35	10YR4/6	absent	4	weak	friable	0.0	0.7	2.3
	Bw	100	10YR5/6	2.5y6/4, 1%	4	moderate	friable	1.3	2.7	2.0
	BC	130+	10YR6/8	10yr7/2, 1%	30	massive	friable	0.3	1.0	1.7
13	Ap1	30	10YR4/4	absent	12	moderate	resistent	0.0	0.3	0.3
	Ap2	75	10YR5/6	10YR5/1, 25%	3	moderate	resistent	1.3	1.0	1.3
	Bkg	110	10YR5/6	10YR5/1, 25%	0	moderate	resistent	2.0	1.7	1.7
	CBg	120+	10YR4/2	2.5Y4/0, 40%	0	massive	very resistent	0.0	0.0	0.0

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Table 2 – Soil chemical and physical characteristics.

Site (BRO)	Horizon	Texture Sand (dag kg^{-1})	Texture Clay (dag kg^{-1})	BD (g cm^{-3})	pH	EC µS cm^{-1}	CaCO3 Total (dag kg^{-1})	CaCO3 active (dag kg^{-1})	OC (g kg^{-1})	N total (g kg^{-1})	AWC (g 100g^{-1})	CEC (meq 100 g^{-1})
1	Ap	22	37	1.1	7.6	454	26.3	7.1	13.6	1.2	9.8	17.5
	Bw	17	42	1.5	8.2	312	25.5	6.8	8.7	0.9	11.4	17.3
	BC	30	35	n.d.	7.9	400	30.8	7.4	10.4	0.8	8.0	16.2
2	Ap	26	39	1.4	8.3	176	25.3	4.5	8.1	0.8	9.0	21.1
	Bw	30	42	1.1	8.3	473	27.5	5.6	6.1	0.7	8.9	20.3
	BC	28	43	n.d.	7.9	340	27.5	5.6	n.d.	n.d.	n.d.	n.d.
4	Ap1	48	29	1.3	7.7	316	2.6	2.5	9.9	0.8	9.2	15.3
	Ap2/A	48	31	1.2	8.4	249	1.6	1.2	7.5	0.7	11.4	14.7
	Ap2/Bw	49	26	1.2	7.6	200	1.1	0.5	4.3	0.3	11.9	12.7
	Bwg	50	25	1.3	7.6	302	1.1	1.0	4.3	0.4	13.0	13.5
5	Ap	26	48	1.5	8.4	482	2.4	1.2	5.8	0.6	11.6	18.0
	Bwg	24	52	1.4	8.5	414	1.1	1.0	3.1	0.4	13.2	17.5
	Cg1	24	44	n.d.	8.3	300	0.9	0.7	4.4	0.5	13.0	14.1
6	Ap1	39	36	1.4	8.7	239	2.5	1.3	6.9	0.5	7.1	14.8
	Ap2/A	43	33	1.5	8.0	292	2.2	1.3	9.8	0.6	6.1	15.6
	Ap2/Btg	45	34	1.6	7.8	286	3.4	1.3	5.4	0.4	10.7	14.7
	Btg	54	27	n.d.	7.7	401	0.9	0.7	3.7	0.3	9.8	16.5
7	Ap1	28	40	1.2	8.0	477	1.8	1.6	6.3	0.5	11.5	23.8
	Ap2/A	28	40	1.5	8.0	137	7.4	2.1	9.7	0.8	9.5	23.4
	Ap2/Bw	18	52	n.d.	8.4	337	4.4	2.4	3.9	0.4	11.2	19.9
9	Ap	48	29	1.4	7.8	221	0.6	0.3	8.8	1.0	9.2	16.4
	Bw	46	30	1.6	8.5	200	0.5	0.4	n.d.	n.d.	n.d.	22.3
	2Bt	22	42	n.d.	8.2	198	0.5	0.4	2.4	0.5	12.0	22.3
10	Ap	46	32	1.5	7.7	124	1.1	0.7	2.6	0.4	7.6	15.9
	2Bt	26	39	1.4	8.2	189	4.6	2.0	5.6	0.6	8.7	17.1
11	Ap	52	20	1.5	8.1	124	15.6	3.0	6.5	0.7	9.2	10.7
	Bw	60	17	1.3	8.4	276	17.3	3.3	2.8	0.4	6.5	9.8
	BC	58	17	1.4	7.6	325	10.9	2.2	1.8	0.2	8.4	9.5
12	Ap	72	10	1.2	8.4	473	6.4	1.1	0.2	0.2	6.9	7.7
	Bw	70	12	1.3	7.8	513	5.5	1.2	0.1	0.2	7.0	7.9
13	Ap1	46	32	1.2	7.8	416	17.8	2.7	44.4	1.4	7.5	29.4
	Ap2	n.d.	n.d.	1.3	n.d.	n.d.	9.2	1.3	16.5	0.9	12.3	15.1
	Bkg	16	45	1.4	8.2	612	36.3	5.5	17.1	0.8	10.2	18.5
	CBg	16	54	n.d.	8.2	270	15.2	3.1	11.8	0.6	10.6	12.4

BD, bulk density; EC, salinity; $CaCO_3$, calcium carbonate; OC, organic carbon; AWC, available water capacity; CEC, cation exchange capacity; n.d., not detected.

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clays with some sandy-gravelly lenses, sometimes with lignite residue and gypsum crystal in the parent material (sites BRO5 and BRO13). The soil is poorly structured or massive, especially in depth, where it often shows redoximorphic features (redox mottles and Fe-Mn nodules). The $CaCO_3$ and the organic matter content are both low, the pH is sub-alkaline (8.3-8.5), and the AWC is moderate to high (130-170 mm m^{-1}). The soil was classified Endogleyic Stagnosol (Eutric, Clayic). Leccio 1, a Cutanic Luvisol (Ruptic, Hypereutric), and Leccio 2, a Brunic Arenosol (Calcaric, Eutric), were both situated on marine sands and conglomerates (sites BRO9 and 10 for LEC1, BRO11 and 12 for LEC2). Leccio 1 is a rather preserved soil and it is placed on stable surfaces or in impluvia. Sometimes it buries an older soil developed on the tertiary flysch. It is reddish in colour, deep, with sandy-clay loamy texture, and common medium and fine gravels. It has scarce $CaCO_3$ (0.5-4.5 %) and exchangeable potassium (48-143 mg kg^{-1}), good drainage and a moderate AWC (120-130 mm m^{-1}). Leccio 2 is the most eroded soil on the marine sands, brownish in colour, poorly structured or loose, loamy sandy textured and with variable gravel content. The $CaCO_3$ is moderate (5-15 %), the organic matter is low (0.4-1.1 % in the Ap horizon), drainage is excessive and the AWC is low to moderate (90-110 mm m^{-1}). Santa Lucia, a Stagnic Cambisol (Calcaric, Clayic), is formed on the relatively more recent Plio-Pleistocene fluvio-lacustrine clays (sites BRO7). It is a brown soil, loamy-clay textured, moderately structured, showing sometimes ferric nodules or a petroferric horizon in depth (about 1-1.5 m deep). This soil is usually poorly gravelly, plastic and with a firm consistency when dry. The $CaCO_3$ is moderate (1.8-12 %) and the organic matter is scarce (1-1.2 % in the Ap horizon). The soil is somewhat poorly drained and has moderate AWC (130-150 mm m^{-1}). All the six studied soils were fully bases saturated, but not saline, and showed low electro-conductivity values (<500 µS cm^{-1}). Nitrogen content was always low or very low, with relatively higher values in sites BRO1, BRO9, and BRO11, and lowest values in the very sandy and eroded site BRO12.

2. Field analysis

The vegetative behaviour of the grapevines was recorded at specific phenological phases and yield components were measured at harvest (yield per vine, cluster and mean berry weight (MBW), number of clusters per vine). Ψstem was monitored at veraison (first days of August) with a pressure chamber, according to the methodology proposed by Choné *et al.* (2001). Measurements were performed between 10 and 11 am on non-transpiring mature leaves that had been bagged before measurement. Water availability or deficit, expressed as the difference between measured soil water content and wilting point, was evaluated during the veraison-harvest phases. Monitoring of the soil water content was performed by FDR (Frequency Domain Reflectometry) sensors at 11 different sites, at intervals of about 15 days over 3 growing seasons (2008-2010). Each site had 3 FDR replicated measurement points, placed under the same vine row at a distance of about 5-6 m apart. Two reference depths (5-35 and 35-75 cm) were selected according to literature (White, 2003). Moreover, for each site the 3-year average water content of the 5-75 cm depth layer, related to the veraison-harvest period, was calculated. Data for the sites BRO2, BRO9 and BRO10 were incomplete, due to damage to the monitoring wells.

3. Laboratory analysis

The isotopic ratio $^{13}C/^{12}C$ ($\delta^{13}C$) was measured in the wine ethanol by Isotope Ratio Mass Spectrometry. The $\delta^{13}C$ was expressed in parts per thousand variations, referred to the international standard (V-PDB) ratio (Farquhar *et al.*, 1989). Wines were analysed twice.

Traits of grape quality (sugar content, sugar accumulation rate, titratable acidity, total and extractable anthocyanins and polyphenols) were analysed in the berries and the musts according to OIV (2000) and Glories (2001). YAN was determined with the enzymatic-spectrophotometric method (Foodlab, 2013) and measured in the grape musts from the different experimental vineyards in the year 2008-2010, or plots in the year 2011.

Routine soil analysis of the air-dried < 2 mm fraction followed the official Italian methods (MIPAF, 2000). Laboratory analyses were carried out on each soil horizon up to the rooting depth. Soil texture was tested in the laboratory by the sieve-pipette method. $CaCO_3$ content was measured volumetrically by the addition of HCl in a Dietrich-Fruhling calcimeter. Active $CaCO_3$ was analysed with a solution of ammonium acetate; this is the more active fraction of $CaCO_3$, which easily dissolves and precipitates. Soil organic carbon content was determined by using the Walkley-Black procedure (Walkley and Black, 1934); pH and electrical conductivity were measured in a 1:2.5 (w w^{-1}) water suspension; cation exchange capacity (CEC) was measured by use of 1 M sodium acetate solution at pH 7.0; and exchangeable bases were extracted with 1 M NH^{4+} acetate solution at pH 7.0 and measured by flame photometry (Na, K, and

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Ca) and atomic absorption spectrometry (Mg). Bulk density (BD) was obtained with the core method (Robertson and Paul, 2000), averaging two or three replicate samples. AWC was estimated as the difference between soil water content at field capacity (FC) and wilting point (WP). Soil moisture content at -33 kPa (FC) and -1500 kPa (WP) of disturbed samples of the benchmark profiles were analysed in laboratory by the pressure-plate system (Kassel and Nielsen, 1986) on replicated samples. The moisture content at FC and WP (mm m^{-1}) were measured on the fine earth fraction and the values were corrected for the gravel content according to Gardner (1986).

4. Climatic and bioclimatic elaborations

ET_0 was estimated according to Hargreaves and Samani (1982) with the aim to compare the climatic conditions of the 4-year study period with the long-term averages. The ombrothermic diagram of Bagnouls and Gaussen (1953) was adopted to characterize the climate of the year and highlight the occurrence of arid conditions. The monthly precipitation and the mean monthly temperature were plotted on the same graph, doubling the values on the scale of precipitation; the dry months are those where the rainfall curve goes below that of temperature, indicating the inversion of the hydric balance. A month is considered "dry" when the value of the sum of the monthly rainfall is equal to or less than twice the value of the average monthly temperature ($P \leq 2T$).

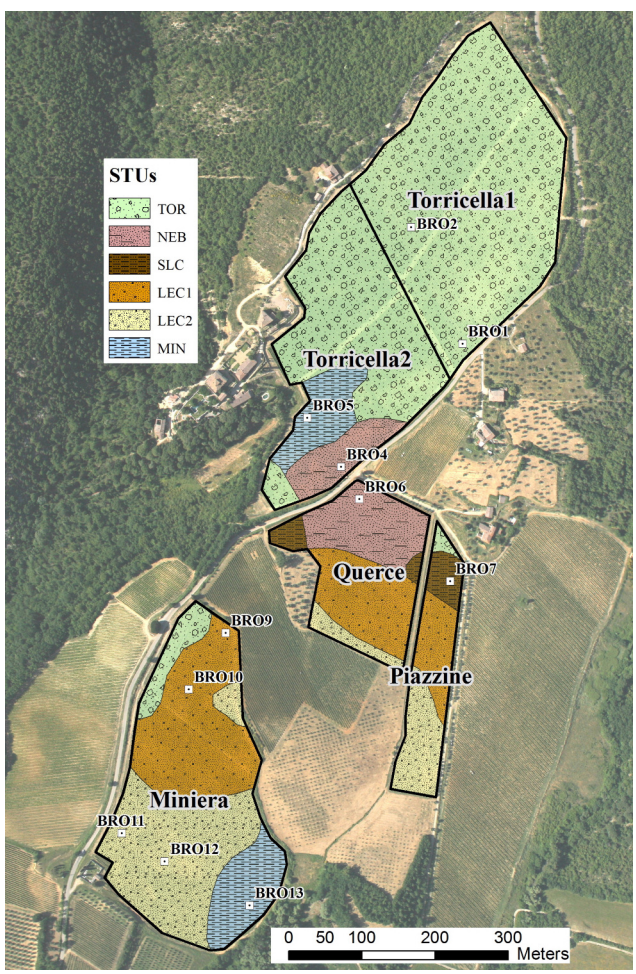


Figure 1 – Soil map of the five experimental vineyards and eleven sites (BRO1-2, BRO4-7 and BRO9-13). Soil typological units (STUs): Nebbiano (NEB), Torricella (TOR), Miniera (MIN), Santa Lucia (SLC), and Leccio 1 and 2 (LEC1 and LEC2). For more details, please see M&M and Tables 1 and 2.

The availability of daily data of solar radiation, maximum and minimum temperature, average wind speed and average temperature of the dew point allowed the estimation of ET_0 according to Penman and Monteith (ET_{0PM}; ASCE-EWRI, 2004). This parameter was used to estimate crop evapotranspiration (ETc) according to Allen *et al.* (1998):

$$ETc = ET_{0PM} \times Kc$$

Kc is the crop coefficients for the vine. The adopted monthly values (Doorenbos and Kassam, 1979) were different along the growing season (0.45 April, 0.65 May, 0.70 June, 0.75 July and August, 0.70 September) to take into account the vine phenological phases and the soil coverage. The climatic water deficit (WDc) for the vine was calculated from April 1 to September 30, as follows:

$$WDc = P - ETc$$

where P is the monthly rainfall.

Two specific bioclimatic indices were also used to characterize the viticultural environment:

The growing degree-days or Winkler index (IW; Winkler, 1962) was calculated using the following formula:

$$IW = \sum_{01/04}^{31/10} (Tmed - 10)$$

where *Tmed* is the mean daily air temperature (°C). The index was calculated for the period from April 1 to October 31.

The Heliothermal index of Huglin (IH; Huglin, 1986) was calculated as follows:

$$IH = \sum_{01/04}^{30/09} \frac{(Tmed - 10) + (Tmax - 10)}{2} \times K$$

where *Tmed* is the mean daily air temperature (°C), *Tmax* is the maximum daily air temperature (°C), and *K* is the length of day coefficient (range from 1.02 to

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1.06 between 40° and 50° of latitude, 1.03 at Brolio); in the northern hemisphere, the index is calculated based on the period from April 1 to September 30.

5. Sangiovese performance

The vine performance of Sangiovese was evaluated by means of a synthetic indicator worked out by Bucelli *et al.* (2010), which makes use of a selected set of vegetative and productive parameters to estimate the score obtained in the wine panel test, as follows:

$Y = 8.689\ °Brix - 141.881\ °Brix\ d^{-1} + 0.042\ EPI + 16.546\ MBW - 170.646$

where Y is the score at the wine panel test, °Brix is the berry sugar content at harvest, °Brix d^{-1} is the sugar accumulation rate, EPI is the extractable polyphenol index from berry skin, and MBW is the mean berry weight at harvest.

The equation, validated for the Sangiovese variety cultivated in the province of Siena, tells us that the wine score obtained in a specific year and plot is higher when the sugar content in berries at harvest is high, the sugar accumulation is slow, the polyphenol content in the berry skin is high, and berries are not stunted.

6. Statistical analysis

Data were submitted to analysis of variance and regression analysis, using Statistica software (StatSoft Inc., Tulsa, OK, USA).

Table 3 – Climatic indices and rainfall during the growing season of the 4 years of study.

Year	Winkler index	Huglin index	April - September rain (mm)
2008	1750	2279	332
2009	1969	2566	266
2010	1721	2282	437
2011	1943	2597	145
Long term	1856	2181	358

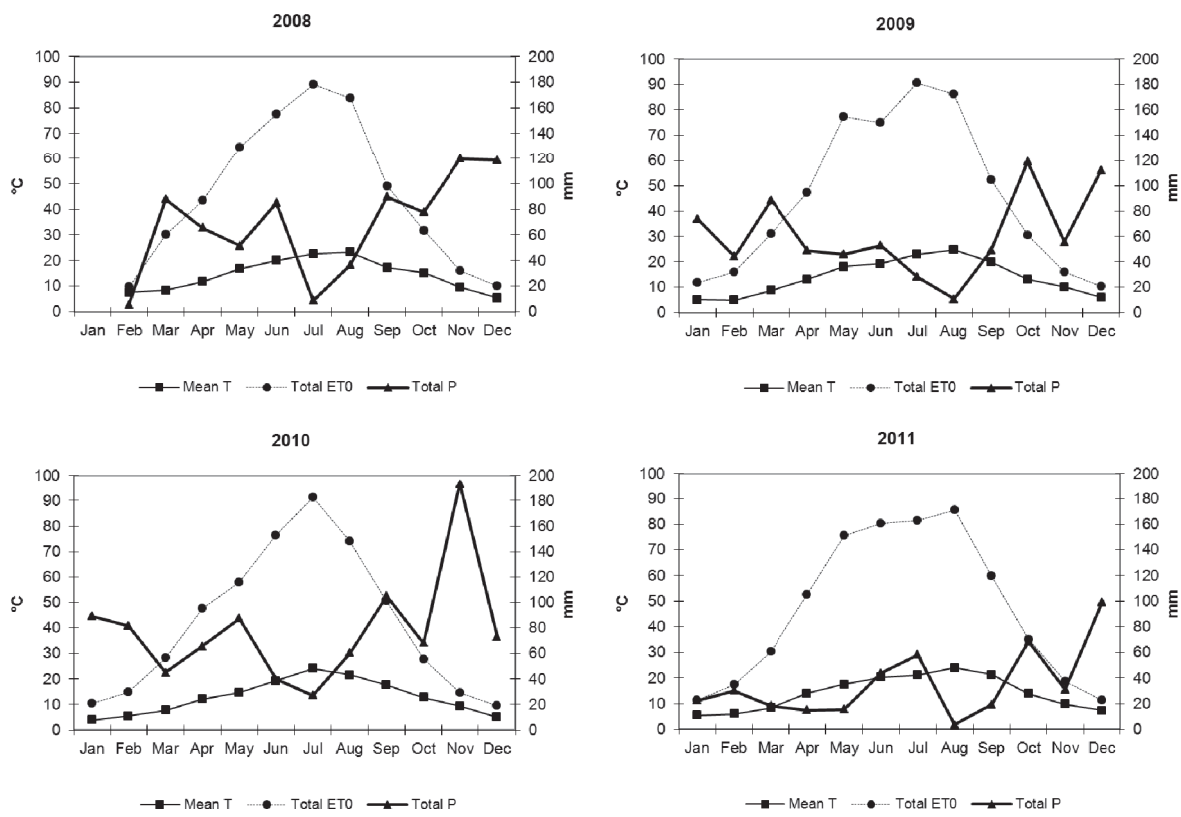


Figure 2 – Ombrothermic diagrams of the studied years (T temperature, ET_0 evapotranspiration, P precipitation).

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RESULTS AND DISCUSSION

1. Climatic conditions during the study

The weather during the study was quite different each year, and in the years 2010 and 2011 compared to long-term average (Figure 2). The year 2010 was the coldest and wettest: annual precipitation was 936 mm and seasonal ET_0 was 795 mm, whereas 2011 was the warmest and driest: annual precipitation was 424 mm and seasonal ET_0 was 870 mm.

The ombrothermic diagrams (Figure 2) highlighted that in the year 2008, which is the most representative of long-term climate, drought conditions occurred at the beginning of July and persisted until mid-August, with July being the driest month; the year 2009 was characterized by a longer period of "drought", which started at the beginning of July and continued until mid-September, with August being the driest month; the year 2010 showed moderate drought conditions that occurred from mid-June until the end of July; finally, the year 2011 was very dry, arid conditions occurred from mid-March until mid-June, and then again from the beginning of August until the end of September.

In the year 2008, the maximum ET_0 occurred in July and August, roughly coinciding with the period of maximum relative aridity. In 2009, ET_0 values as high as those recorded during the «drought» period were also estimated in May. In 2010, the peak values of July were lower than in the two previous years. In 2011, there were high values of ET_0 from May until August.

Meteorological conditions during the growing period in the 4 years of study were also characterized by Huglin and Winkler indices through the data of the weather station placed in the vineyards and compared with the long-term data recorded at the nearby station of Gaiole in Chianti. They were slightly lower than the long-term average in 2008 and 2010, whereas they were higher in 2009 and 2011 (Table 3).

Rainfall during the growing period in 2009 and 2011 was lower than the long-term average (316 mm) and higher in the year 2010. In 2009, rainfall was concentrated in the post-flowering and pre-harvest periods, while in 2008 total rainfall, showing an amount similar to that of the long-term average, was concentrated in a few heavy downpours; by contrast, in 2010 rainfall was evenly distributed throughout the summer, with greater intensity than in the previous two years. In 2011, rainfall during the growing season was scarce and concentrated in June and July (data not reported).

The climatic water deficit, estimated during the vine growing season on a monthly basis, further highlighted the differences between the years of study. A pronounced water deficit occurred in 2009 and was even more pronounced in 2011, when the vine was always under potential water deficit conditions. The cumulative potential climatic deficit from July to September was -68.8 mm in 2008, -114.7 mm in 2009, 36.7 mm in 2010, and -120.2 mm in 2011 (Figure 3).

2. Soil water availability

The water availability for the period between the beginning of July and the harvest, which is considered critical for the accumulation of sugars and the development of secondary metabolites in the grapes, is shown in Figure 4. The figure shows the difference between mean field soil water content and lab water content at a tension of -1,500 kPa (standard wilting point) during the period from veraison to harvest. The presence of negative values can be explained recalling that vines are able to absorb water from depths greater than that monitored and at tensions stronger than -1,500 kPa (White, 2003).

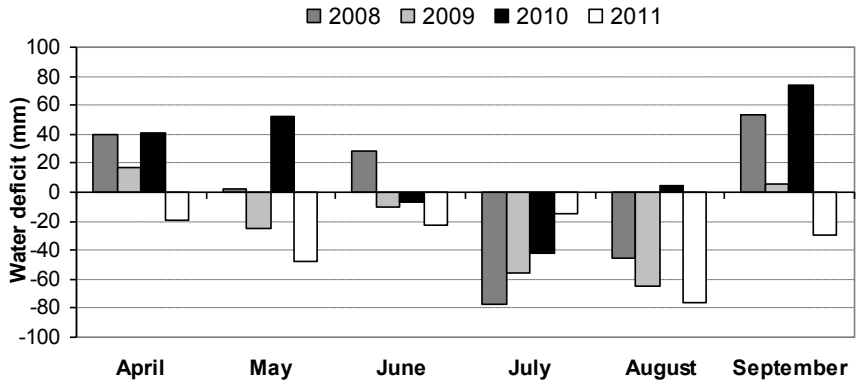


Figure 3 – Climatic water deficit for vine in the four study years.

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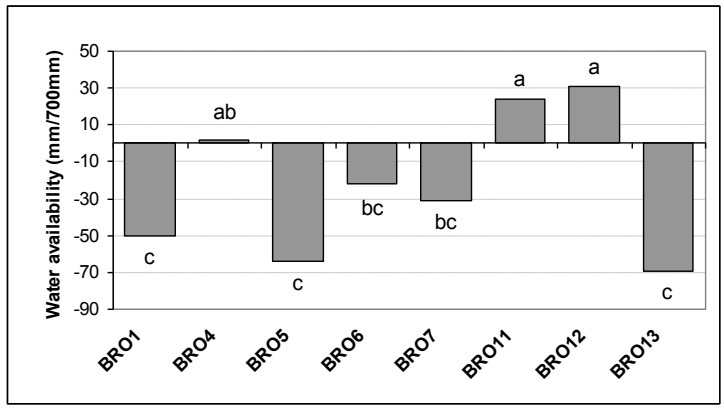


Figure 4 – Mean soil water availability in the 5- to 75-cm depth between the beginning of July and the harvest (2008-2010). Bars marked with different letters are significantly different for P < 0.05 according to the HSD Tukey's test.

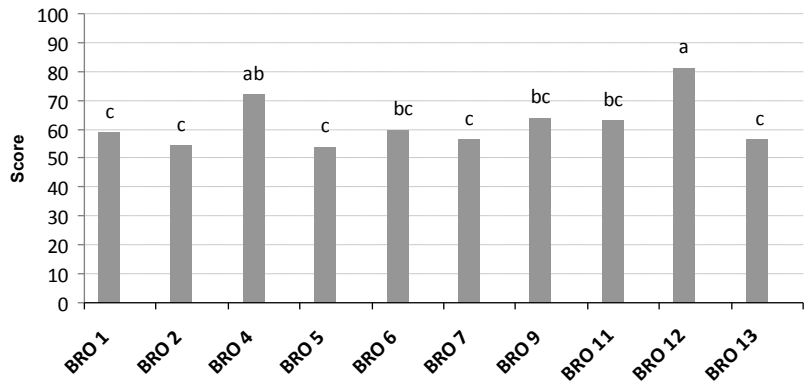


Figure 5 – Mean values of Sangiovese performance in the studied sites (2008-2010). Bars marked with different letters are significantly different for P < 0.05 according to the HSD Tukey's test.

Despite the considerable climatic differences that occurred in the first three years, the statistical analysis of the mean soil moisture did not reveal significant differences between years (about -18 mm on average). On the other hand, there was a high variability between sites, and each site showed similar behaviour throughout the monitoring period. In fact, significant differences emerged among the different monitoring sites.

It is not surprising that a positive difference occurred in sandy soils (BRO11 and BRO12), whereas clayey soils showed the most negative values (BRO1, BRO5 and BRO13). Actually, water retention capacity of the studied soils was not only affected by the texture, but also by soil structure, gravel, and stone content. Therefore, moisture in poorly structured or very stony clayey soils did not translate into a larger volume of water effectively present in the soil and available to the vines.

3. Sangiovese performance

Grape production during the studied years was characterized by rather low values (general average of 1.66 kg per plant), as pursued by the farm, but without any statistical relationship with the viticultural results (Table 4) and the calculated performance. On the other hand, sites showed statistically significant differences for some of the other parameters, in spite of the uniform and severe pruning (Table 4). The effect of the agricultural husbandry was also evidenced by the absence of a significant difference between years in terms of mean berry weight and yield. On the contrary, the effect of year was significant on sugar content and accumulation, pointing to higher values in 2010, and in particular on polyphenol content.

The score of the mean three-year Sangiovese performance obtained in the studied sites was always above 50, which means that in all plots the quality potential of the grapes was good. The performance ranged significantly between years (on average, 58 in 2009 versus 64 in both 2008 and 2010), and especially between sites (Figure 5). Sites BRO12 and BRO4 showed significantly higher performances compared to the others, especially compared to BRO1, 2, 5, 7 and 13.

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Table 4 – Main viticultural results.

Site	year	Sugar content °Brix	Sugar accumulation rate °Brix day^{-1}	MBW* g	Yield per plant kg	Extractable polyphenol index mg kg^{-1}
BRO 1	2008	20.12	0.33	1.95	1.72	1675
	2009	20.60	0.34	1.79	1.45	1465
	2010	21.38	0.39	2.06	1.46	1790
	mean	20.70 **b**	0.35 **bc**	1.93 **bc**	1.54 **b**	1643 **ab**
BRO 2	2008	21.85	0.36	1.95	1.52	1423
	2009	21.43	0.35	1.90	1.62	1352
	2010	21.00	0.38	1.92	1.20	1731
	mean	21.43 **ab**	0.36 **abc**	1.92 **bc**	1.45 **b**	1502 **ab**
BRO 4	2008	22.20	0.36	2.07	1.51	1640
	2009	22.30	0.37	2.49	1.24	1399
	2010	22.40	0.41	2.22	1.47	1947
	mean	22.30 **ab**	0.38 **ab**	2.26 **ab**	1.41 **b**	1662 **ab**
BRO 5	2008	19.63	0.32	2.03	1.61	1677
	2009	19.70	0.32	2.17	1.95	1165
	2010	20.97	0.38	2.17	1.73	1727
	mean	20.10 **b**	0.34 **c**	2.12 **abc**	1.76 **ab**	1523 **ab**
BRO 6	2008	21.40	0.35	1.97	1.28	1536
	2009	21.07	0.35	2.04	2.22	1232
	2010	22.85	0.42	2.09	n.d.	2016
	mean	21.77 **ab**	0.37 **abc**	2.03 **abc**	1.75 **ab**	1595 **ab**
BRO 7	2008	21.92	0.36	2.02	1.69	1287
	2009	21.70	0.36	1.90	n.d.	1336
	2010	22.76	0.41	2.22	1.70	2003
	mean	22.13 **ab**	0.38 **abc**	2.05 **abc**	1.70 **ab**	1542 **ab**
BRO 9	2008	22.01	0.36	2.27	2.85	1515
	2009	20.70	0.34	2.58	2.43	1433
	2010	20.71	0.38	2.27	n.d.	1687
	mean	21.14 **ab**	0.36 **abc**	2.37 **a**	2.64 **a**	1545 **ab**
BRO 10	2008	n.d.	n.d.	n.d.	n.d.	n.d.
	2009	19.96	0.36	n.d.	n.d.	1244
	2010	22.88	0.40	n.d.	n.d.	n.d.
	mean	21.42 **ab**	0.38 **abc**	-	-	1244 **b**
BRO 11	2008	20.85	0.34	2.15	2.25	1496
	2009	22.50	0.37	1.97	1.67	1337
	2010	23.26	0.42	2.01	1.06	2080
	mean	22.20 **ab**	0.38 **abc**	2.04 **abc**	1.66 **b**	1638 **ab**
BRO 12	2008	23.35	0.38	1.76	0.75	1837
	2009	23.00	0.38	1.97	1.12	1722
	2010	23.32	0.42	1.84	1.51	2089
	mean	23.22 **a**	0.39 **a**	1.86 **c**	1.13 **b**	1883 **ab**
BRO 13	2008	19.74	0.32	2.09	1.83	1528
	2009	19.70	0.32	2.08	1.23	1513
	2010	22.33	0.41	2.01	0.89	1937
	mean	20.59 **b**	0.35 **bc**	2.06 **abc**	1.32 **b**	1659 **ab**
Year	*2008*	*21.31* ***b***	*0.35* ***b***	*2.03* ***a***	*1.70* ***a***	*1561* ***b***
	2009	*21.15* ***b***	*0.35* ***b***	*2.09* ***a***	*1.66* ***a***	*1382* ***c***
	2010	*22.17* ***a***	*0.40* ***a***	*2.08* ***a***	*1.38* ***a***	*1901* ***a***
	mean	*21.54*	*0.37*	*2.07*	*1.58*	*1615*

*MBW, mean berry weight and n.d., not detected; in each column, mean values with different letters are significantly different for $P < 0.05$ according to the HSD Tukey's test.

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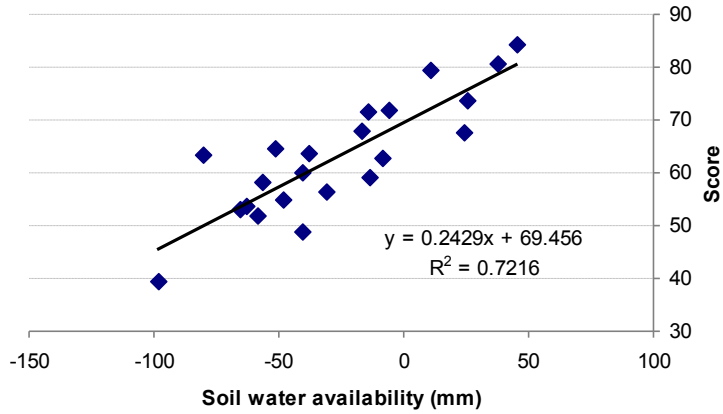


Figure 6 – Data plotted from Sangiovese performance and soil water availability in the 5- to 75-cm depth in seven sites during the years 2008-2010. Negative values indicate mean soil water contents below conventional wilting point at the measured depth between veraison-harvest phases.

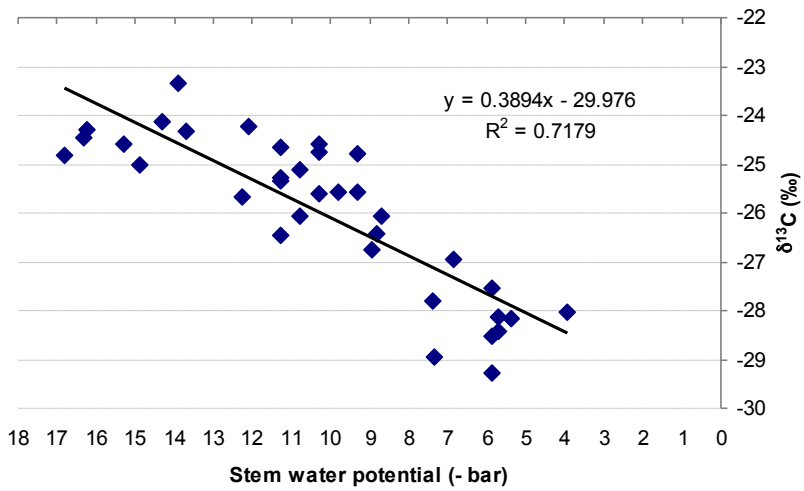


Figure 7 – Data plotted from $\delta^{13}C$ and stem water potential measured at veraison in four (2010) or eight (2011) sites.

It is worthwhile to note that the Sangiovese performance in the studied plots was positively related to soil water availability at 5- to 75-cm depth, with a rather high determination coefficient (Figure 6). This outcome was particularly interesting, since previous studies had indicated that the viticultural result of Sangiovese is generally limited by large water availability, as it causes superfluous growing at the expense of the target grape quality (Storchi *et al.*, 2005; Scalabrelli *et al.*, 2006; Costantini *et al.*, 2008; Palliotti *et al.*, 2011).

4. Carbon isotopes measured on wine ethanol

As expected, $\delta^{13}C$ determined in the wine samples was rather well correlated with Ψstem (Figure 7). The Ψstem threshold of -12 bar, reported in the literature to separate stress from no stress water conditions, corresponded to a $\delta^{13}C$ value of -25.3‰ (broadly equivalent to -23.7‰ in the must, according to Rossmann *et al.*, 1996). The value was slightly lower than that reported in the literature for red wines, but matched well with the outcomes obtained from Sangiovese cultivated in different experimental settings (Costantini *et al.*, 2010; Palliotti *et al.*, 2008).

In few cases, the measured Ψstem values were lower than -12 bar, but most values were lower than -9 bar, corresponding to a $\delta^{13}C$ value of -26.5‰. If we take -9 bar Ψstem as a threshold for Sangiovese to point to the occurrence of incipient water stress, the results indicated that many of the observed plants were suffering from moderate or severe water stress at the date of measurement.

As a whole, the general mean $\delta^{13}C$ value obtained in the wines of all plots and years, -25.48‰ (-23.78‰ in the must), pointed to an average water stress. Significant differences occurred between years as well as between sites. Limiting water nutrition was

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stronger in 2008, moderate in 2009, and low in 2010 (Table 5).

Means with different letters indicate significantly differences between sites in the same year for $P < 0.05$, or between year averages for $P < 0.01$, according to the HSD Tukey's test.

As for the influence of year, it is noteworthy that there was no clear relationship between $\delta^{13}C$ values and climatic deficit during the entire growing season, the veraison-harvest phases (Table 5), or the Winkler and Huglin indices. However, in the wettest year (i. e., 2010) we obtained the most negative $\delta^{13}C$ values, and the amount of climatic deficit during July, namely the time before full veraison, seemed to have a trend similar to that of $\delta^{13}C$ values.

On the other hand, sites showed a similar ranking in all years, in other words, no statistical interaction between the effects of the site and year occurred. This means that there was a clear differentiation of the three-year mean values between sites, in particular, BRO4 and BRO12 showed the lowest values, indicating no or very little water stress, while all the others were under the threshold of moderate stress (-26.5‰), with BRO1, BRO2, BRO5, BRO6, BRO7, BRO10, and BRO11 under the threshold of strong stress (-25.3‰) (Figure 8).

The most interesting outcome of the isotopic characterization of the wines, however, was achieved when $\delta^{13}C$ values were related to the viticultural and oenological results, as synthesized in the performance index (Figure 9). In this case, the correlation was contrary to expectation, that is, the performance increased when the water stress decreased. This result was particularly striking since $\delta^{13}C$ values were not particularly low, and could confirm a particular sensitivity of the Sangiovese performance to water limitations, similar to those caused by an excess of humidity. Thus, Sangiovese exhibited high adaptation but also sensitivity to dry conditions.

Table 5 – $\delta^{13}C$ values in the studied years 2008, 2009, 2010.

Site	2008	2009	2010
BRO 1	-24.13 bc	-23.35 e	-25.02 c
BRO 2	-24.27 bc	-24.18 cde	-25.88 bc
BRO 4	-26.90 a	-27.62 ab	-28.24 a
BRO 5	-25.17 b	-24.48 cde	-25.74 bc
BRO 6	-23.92 c	-23.66 de	-24.83 c
BRO 7	-23.97 c	-25.02 c	-26.40 b
BRO 9	-24.32 bc	-26.85 b	-27.90 a
BRO 10	-24.24 a	-24.45 cde	-25.70 bc
BRO 11	-23.84 c	-24.20 cde	-25.22 bc
BRO 12	-27.47 a	-28.88 a	-28.59 a
BRO 13	-24.69 bc	-24.78 cd	-26.36 b
Average	-24.87 C	-25.22 B	-26.35 A

Means with different letters indicate significantly differences between sites in the same year for $P < 0.05$, or between year averages for $P < 0.01$, according to the HSD Tukey's test.

5. Yeast-assimilable nitrogen (YAN)

Similarly to soil nitrogen content, which was always low, YAN measured in the must obtained from the bulk yield of the five experimental vineyards was at all times rather low, that is, much lower than the 120-140 mg L^{-1} threshold usually considered as a reference value to assess limiting condition (Ojeda and Salmon, 2011). On average, the three-year mean of YAN was 86.7 mg L^{-1}, without significant differences between years and rather low differences between vineyards (Figure 10). Then we can assume that nitrogen nutrition was always limiting, although water stress conditions changed significantly between years and sites.

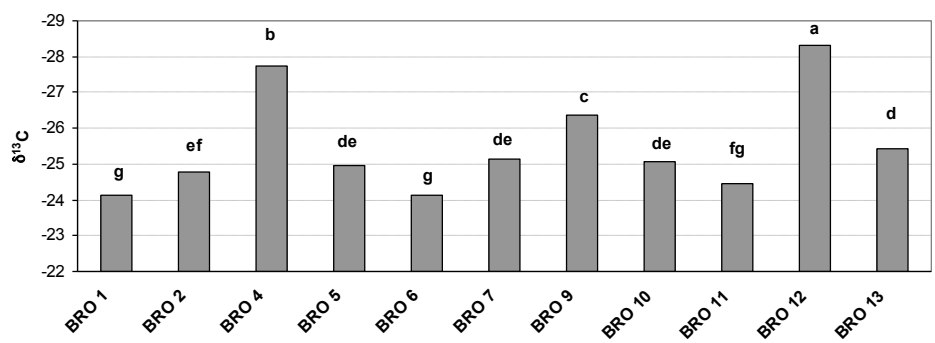


Figure 8 – Mean values (2008-2010) of $\delta^{13}C$ in the wines produced in the experimental plots. Bars marked with different letters are significantly different for $P < 0.05$ according to the HSD Tukey's test.

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6. The validation year

In the year 2011, the overall mean $\delta^{13}C$ indicated moderate water stress conditions, ranging from no (BRO4) to strong stress (BRO1, BRO5, and BRO11) (Table 6). Therefore, the selected subset of plots could be considered well representative of the experimental field variability. Also in this year, $\delta^{13}C$ values did not reflect the seasonal climate (Table 3) and the potential water deficit (Figure 3); rather, they were very much similar to those obtained in the same sites during the previous years (Table 6).

The performance score was on average higher than in previous years, but the site ranking was the same. Although the limited set of data did not allow a significant correlation between the variables, in the year 2011 it resulted in an inverse relationship between $\delta^{13}C$ and performance.

The YAN value was always low to very low. The limited amount of data did not permit generalization, however, there was a direct relation between performance and YAN. Thus, the plot with the worst performance showed the strongest water deficit and

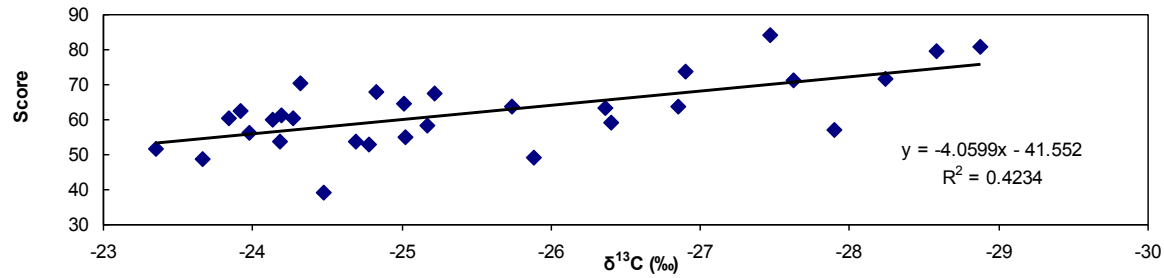


Figure 9 – Data plotted from Sangiovese performance and $\delta^{13}C$ in eleven sites during the years 2008-2010.

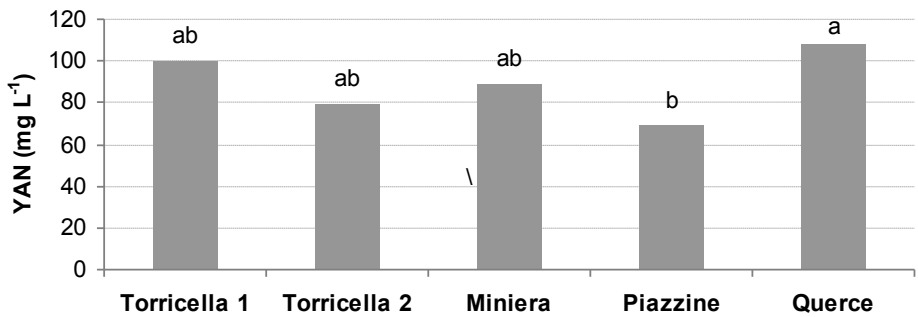


Figure 10 – Mean values (2008-2010) of YAN (Yeast-Assimilable Nitrogen) in the wines produced in the experimental vineyards.
Bars marked with different letters are significantly different for P < 0.05 according to the HSD Tukey's test.

Table 6 – Yeast-assimilable nitrogen (YAN) in the must.

	2011		
Soil	**$\delta^{13}C$ (‰)**	**Performance (score)**	**YAN (mg L^{-1})**
BRO 1	-24.00 c	81.6 ab	104.0 ab
BRO 2	nd	63.0 ab	69.0 ab
BRO 4	-28.12 a	91.6 ab	119.3 a
BRO 5	-24.47 c	58.7 b	57.3 b
BRO 11	-24.58 c	96.1 a	97.0 ab
BRO 13	-25.95 b	71.9 ab	107.7 ab
Average	-25.42	77.1	92.4

Means with different letters are significantly different for P < 0.05 according to the HSD Tukey's test, n.d. : non detected

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the lowest nitrogen availability, whereas the best performance corresponded to moderate or no water stress and relatively higher nitrogen availability.

CONCLUSIONS

The soil survey of the experimental vineyards revealed a large natural variability of soil environments, increased by the mechanical works carried out during the activities of surface preparation before planting. Besides enhancing site specificity, the deep upsetting of the original soil profile and the stripping of the surface horizons favoured the outcropping of stones and biologically poor, deep soil layers. Therefore, the studied soils were in most cases of low fertility, because of their lithological nature as well as a consequence of pre-plantation activities.

The target oenological result pursued by the farm was the enhancement of wine quality and site specificity by means of limited yield and adoption of organic farming. Viticultural husbandry limited fertilization and implemented high plant density, moderate vigour rootstock, severe pruning and thinning of bunches, and a semi-permanent grass cover.

The values of $\delta^{13}C$ were significantly correlated with leaf water potential. A $\delta^{13}C$ in the wine of -26.5‰ corresponded to a Ψstem of -9 bar, while a Ψstem of -12 bar matched with a $\delta^{13}C$ of -25.3‰. The outcomes of the field monitoring of soil water availability confirmed the crucial role played by water supply on Sangiovese performance, but with a direct relationship, contrary to what is considered a general rule for red wines. In fact, the best performance was achieved where water nutrition was not limited, that is, in the plots where the $\delta^{13}C$ in the wine was lower than -26.5‰ and the Ψstem at veraison higher than -9 bar.

The unexpected relationship between $\delta^{13}C$ values and performance scores could be related to the nitrogen nutrition. Actually, all the studied soils showed low or very low nitrogen content, consequently, nitrogen content in the must was also low or very low. In the plots where water availability was relatively higher, nitrogen plant uptake was favoured, YAN was relatively higher, and so was Sangiovese performance. It must be stressed, however, that the used Sangiovese performance evaluation was a simplified approach, which did not allow to highlight possible particular aromas that might have been produced in very limiting soil conditions.

In conclusion, the $\delta^{13}C$ and Ψstem water stress indicators alone cannot be used to foresee vine performance if another important factor, like nitrogen availability, is limiting. The relationship between $\delta^{13}C$ and Sangiovese performance was not as expected, since poor soil fertility, in particular low nitrogen content, and the adopted viticultural husbandry, always prevented vine luxuriation, also in non-limiting water conditions.

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