BERRY CARBON ($\delta^{13}C$) AND NITROGEN ($\delta^{15}N$) ISOTOPIC RATIO REFLECTS WITHIN FARM TERROIR DIFFFERENCES

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Abstract
The natural abundance of carbon stable isotopes has been reported to be related to water availability in grapevines quite widely. In the case of nitrogen, the natural abundance of its stable isotopes is mainly affected by the nature of the source of nitrogen (organic vs. inorganic) used by the plant, though the bibliography available for grapevine is very scarce. The aim of this work was to evaluate the effect of terroir on carbon and nitrogen stable isotope natural abundance within a single grape growing farm. Three vineyards representative of three terroirs within a grape growing farm were selected. The mesoclimatic differences between them can be considered negligible, and crop management was in general terms the same. Therefore, the differences in plant behaviour should be majorly a consequence of soil characteristics (deep gravely vs. shallower loamy soil, cover crop vs. bare soil). During five consecutive seasons, plant vegetative growth and stem water potential (Ψs) were monitored throughout the growing season and, at harvest, yield and grape composition were determined including carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic ratios. Consistent differences for both $\delta^{13}C$ and δ15N were found when the three *terroirs* were compared. On the one hand, $\delta^{13}C$ reflected well the differences in water availability arising from either soil characteristics (deep gravelly vs. shallower loamy soil) and from the presence of a cover crop. On the other hand, $\delta^{15}N$ was clearly higher in the gravelly soil area, possibly indicating nitrate leakage, since soil organic matter is known to have higher $\delta^{15}N$ than inorganic fertilizers. The competition the cover crop exerted for N was reflected in berry nitrogen content but, on the contrary, did not affect $\delta^{15}N$.

Keywords: natural isotope abundance, water use efficiency, water status, nutrition, nitrogen sources,* Vitis vinifera *L.

1 INTRODUCTION

The natural abundance of carbon stable isotopes (^{12}C and ^{13}C) has been reported to be related to water availability in grapevines quite widely (De Souza et al. 2003; De Souza et al. 2005; Des Gachons et al. 2005; Gaudillere et al. 2002; Gómez-Alonso and García-Romero 2010; Guix-Hébrard et al. 2007; Santesteban et al. 2011; Santesteban et al. 2012; Van Leeuwen et al. 2009). Under field conditions, berries have been shown to be the most representative organ, as their carbon isotope ratio ($\delta^{13}C$) is more related to WUE (De Souza et al. 2005) or to water potential (De Souza et al. 2003; De Souza et al. 2005) than $\delta^{13}C$ in other organs. Concerning temporal sensitivity, Santesteban (2012) compared $\delta^{13}C$ in berry samples picked at harvest with average stem water potential (Ψs) measured at different moments of the season, the highest correlation being found for Ψs between veraison and harvest.

In the case of nitrogen, the natural abundance of its stable isotopes (^{14}N and ^{15}N) is mainly affected by the nature of the source of nitrogen used by the plant, since plant uptake is known not to induce significant fractionation during the absorption process, particularly when the external nutrient concentration is lower than vegetation needs (Billy et al. 2010). Thus, since organic matter usually shows $\delta^{15}N$ values much higher than inorganic fertilizers (Bateman and Kelly 2007), it is possible to estimate -to certain extent- which is the major source of N. There is very little research dealing with $\delta^{15}N$ in grapevines and, to our knowledge, Stamatiadis et al. (2007) is the only work published to date that has evaluated this parameter under field conditions. These authors studied within-field variability observed for leaf $\delta^{15}N$ at two vineyards during two consecutive seasons, using 25 to 32 sampling sites per field. These authors reported that vineyard and season influenced leaf $\delta^{15}N$, but also that relevant within-field differences (from 0.42 to 9.12‰) occurred. The highest values were observed at the upper parts of the field with a greater slope, which was attributed to a greater reliance of the plants at those positions to organic sources of N, due to a greater leakage of the N fertilizer that had been applied in spring. Due to the potential interest of $\delta^{15}N$ for viticultural research, it is necessary to increase our knowledge on how this ratio is affected by environmental factors and agronomic practices.

The aim of this work was to evaluate the effect of *terroir* on carbon and nitrogen stable isotope natural abundance at three *terroirs* within a single grape growing farm.

2 MATERIALS AND METHODS

Three vineyards representative of three *terroirs* within a grape growing farm were selected. The experiment was carried out during five consecutive years (2009-2013). Weather conditions during those seasons are summarised in Figure 1. The grape growing farm considered was located at Traibuenas (42º22' N; 1º37' W; WGS84; 340 m asl), and the three vineyards considered were planted with a Tempranillo/110Richter combination, training system was a bilateral cordon and plant spacing was 3 m (between rows) x 1 m (between plants in a row). At each site, 20 vines, representative of average vine size and homogeneous according to their trunk cross sectional area (TCSA), were marked and used for all experimental measurements.

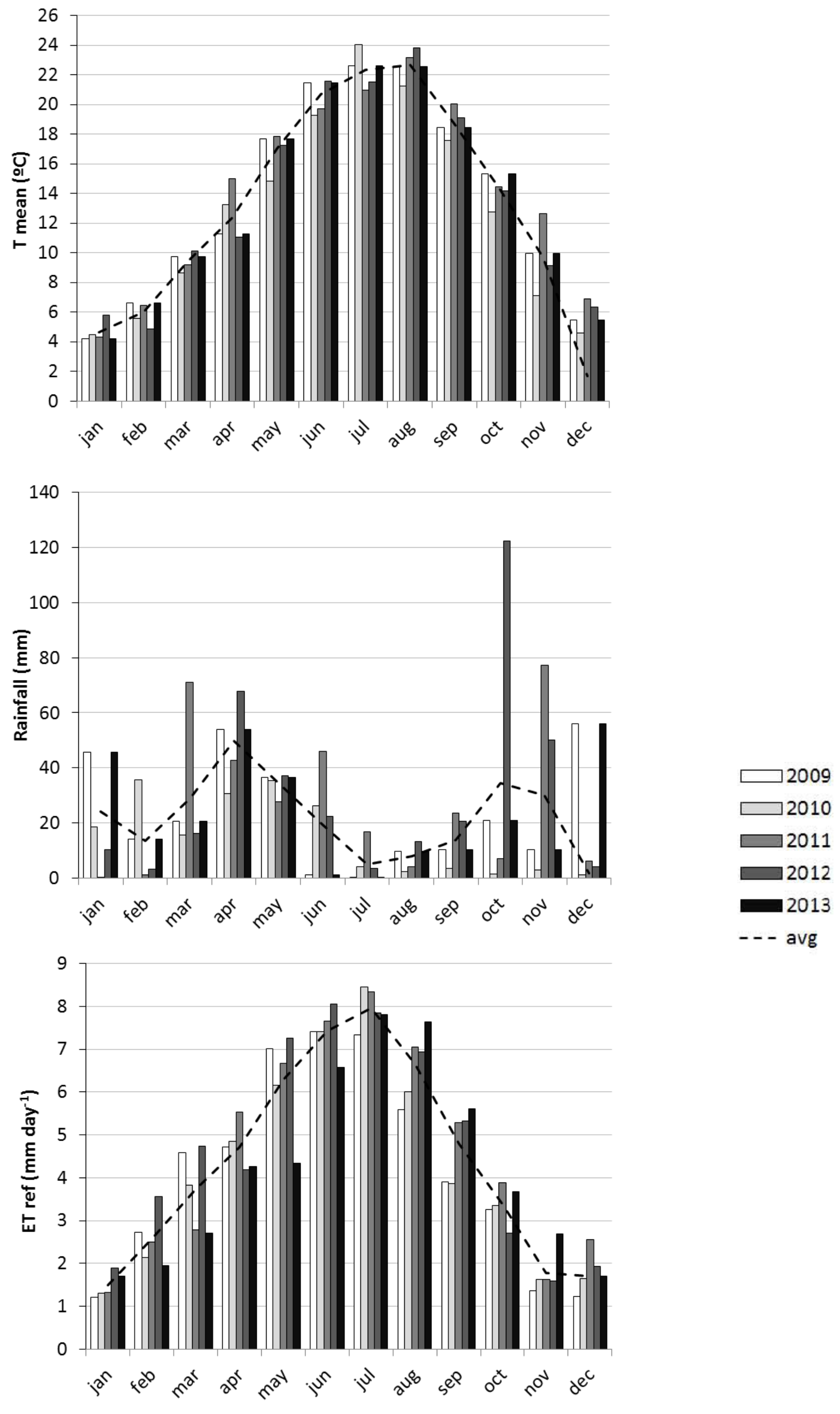

Figure 1: Monthly mean temperature, accumulated rainfall and reference evapotranspiration (ETref) of the five seasons considered in this study

The mesoclimatic differences between the three vineyards can be considered negligible, as they are relatively close to each other (< 1 km), with nearly no differences in altitude (<8 m), and all the three fields show a slight south-facing slope aspect (< 2%). Crop management was in general terms the same, so the differences in plant behaviour should be majorly a consequence of soil characteristics (deep gravely vs. shallower loamy soil, cover crop vs. bare soil). Vineyards have been labelled as Vineyard #1(deep gravely soil), Vineyard #2 (bare loamy soil) and Vineyard #3(cover cropped loamy soil).

Every season (2009-2013), plant vegetative growth and stem water potential (Ψs) were monitored throughout the growing season and, at harvest, yield and grape composition were determined. Stem water potential at midmorning (Ψs-m) was measured from 15 d after fruitset until harvest using, for each vineyard, at least five young healthy leaves taken from five vines that had reached about 2/3 of their definitive size. Measurements were conducted using a Scholander pressure bomb (P3000, Soil Moisture Corp., Santa Barbara, CA, USA). Leaves were bagged 1.5 h prior to measurement using zip-bags covered with a metalized high density polyethylene reflective film (SonocoRF, Sonoco Products Co., Hartsville, SC, USA). When taking leaf samples and performing measurements, the precautions suggested by Turner (1988) were considered.

$\delta^{13}C$ and $\delta^{15}N$ were measured in 100-berry samples randomly picked at harvest. Samples were transferred at cool temperature (7 ºC) to the lab, oven dried at 75 ºC, and ground to a fine homogeneous powder. From each ground sample, three 2 mg subsamples were analyzed using an Elemental analyzer (NC2500, Carlo Erba Reagents, Rodano, Italy) coupled to Isotope Mass Spectrometer (Thermoquest Delta Plus, ThermoFinnigan, Bremen, Germany). Isotope ratios have been expressed as

$$\delta(‰) = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000$$

where R_{sample} and $R_{standard}$ are the heavy-to-light isotope ratio of the sample and the standard respectively (Sulzman 2007). The standards considered were V-PDB (Vienna Pee Dee Belemnite, ^{13}C:^{12}C = 0.0112372) and atmospheric nitrogen (^{15}N:^{14}N = 0.003663).

3 RESULTS AND DISCUSSION

The values observed for $\delta^{13}C$ and $\delta^{15}N$ are presented in Figure 2. $\delta^{13}C$ ranged between -24.5 and -28.2‰, within the ranges reported for this variable in earlier literature for whole berries or must. These values, according to Van Leeuwen et al. (2009), correspond to vines submitted to deficit ranging from moderate to null. Concerning $\delta^{15}N$, the values ranged from 0.2 up to 5.3‰, lower values than those reported by Stamatiadis et al. (2007) when measuring $\delta^{15}N$ in petioles.

Consistent differences for both $\delta^{13}C$ and $\delta^{15}N$ were found when the three vineyards were compared. On the one hand, $\delta^{13}C$ reflected well the differences in water availability arising from soil characteristics, the highest values being observed in vineyard #1 where the gravely soil was generally not able to provide vines with enough water at the end of the season. When vineyards #2 and #3 are compared, the presence of a cover crop in the latter did not appear to cause consistent differences in their water status. Nevertheless, the ability of $\delta^{13}C$ to integrate vine water status is confirmed, since a very significant relationship (R^2 = 0.75, P<0.001) was found when $\delta^{13}C$ and Ψs-m between veraison and harvest were compared (Fig 3a).

On the other hand, as depicted in Figure 2b, $\delta^{15}N$ was found to be clearly higher in the gravelly soil area (vineyard #3), and quite similar irrespective of the presence of the cover crop in the loamy soil (vineyards #2 and #3). An hypothesis that could explain this behaviour would be that nitrate leakage could be more significant at vineyard #1 than at the others which would force vines to rely more on organic sources of nitrogen than on inorganic ones. As soil organic matter is known to have higher $\delta^{15}N$ than inorganic fertilizers, and no fractionation occurs during absorption, higher $\delta^{15}N$ are expected under higher leakage conditions. A similar effect was already alleged by Stamatiadis et al. (2007) to be the cause of observing higher $\delta^{15}N$ at the higher parts of an sloppy vineyard. Conversely, a loose relationship was found (R^2 = 0.27, P=0.003) when N content in berries (estimated from YAN measurements) and $\delta^{15}N$ were compared (Figure 3b), which agrees with the hypothesis of considering $\delta^{15}N$ dependant on N source but not on N availability. Thus, similar $\delta^{15}N$ values were reported independently of the presence of the cover crop or not, despite YAN values were consistently lower in the cover cropped vineyard due to the competition the cover crop exerted for N.

(a) (b)

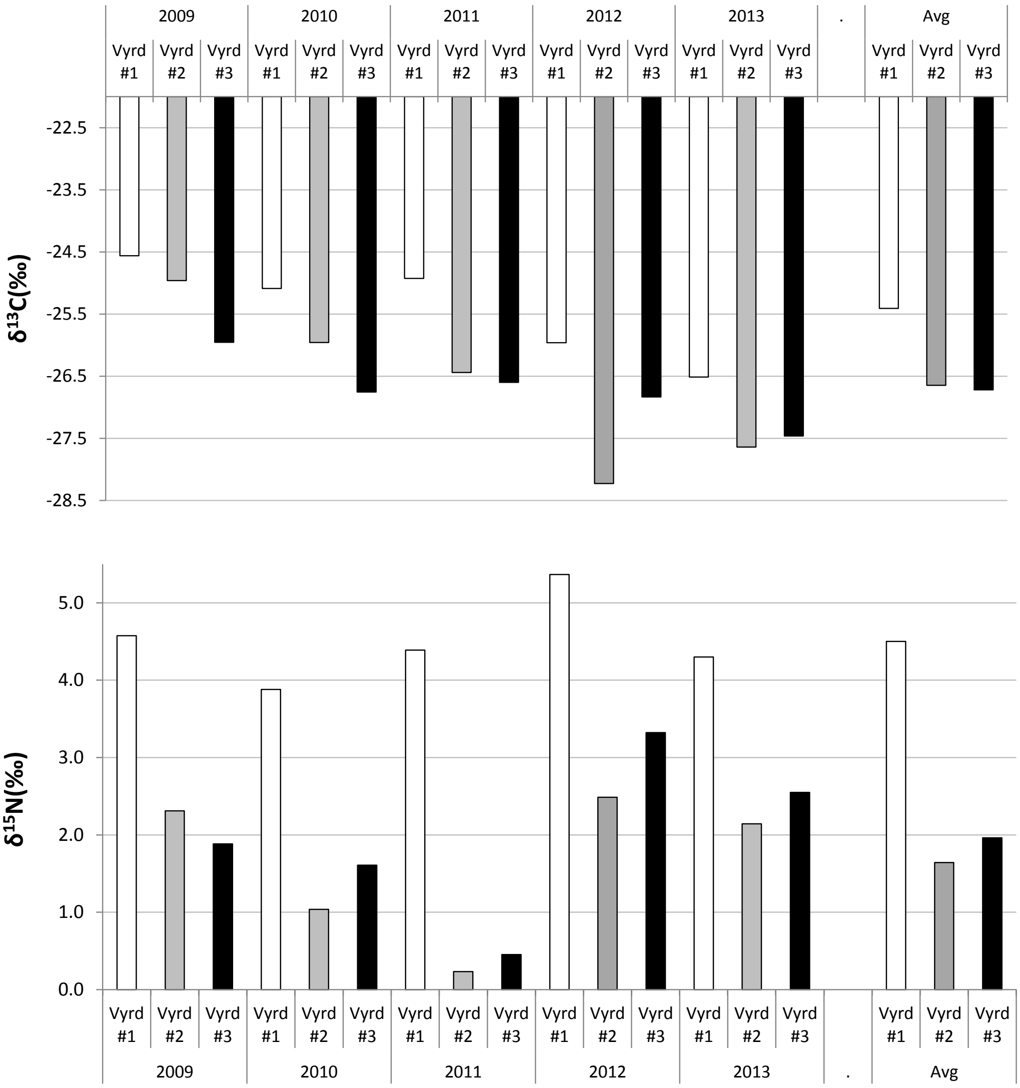

Figure 2: Effect of the year and vineyard considered on (a) carbon ($\delta^{13}C$) and (b) nitrogen ($\delta^{15}N$) isotope ratios of berries at harvest

The results obtained show the potential interest of measuring the natural abundance of carbon and nitrogen stable isotopes for *terroir* characterization and study. Thus, $\delta^{13}C$ was shown to be very well related to vine water status in the last part of the season, as already shown in earlier works (De Souza et al. 2003; De Souza et al. 2005; Gaudillere et al. 2002; Guix-Hébrard et al. 2007; Santesteban et al. 2012; Van Leeuwen et al. 2009). Therefore, it would be possible to use $\delta^{13}C$ to classify grape batches depending of the water deficit experienced in the vineyard with no need of measuring it. As it is widely known, water status can frequently be one of the major drivers of grape quality (Van Leeuwen et al. 2009) and, depending on the season, a greater or lesser level of stress at the end of the season can be required to produce premium grapes.

The study of $\delta^{15}N$ could help us having better information on the origin of a batch of grapes, since terroir has been shown to modify it. Apart from that, $\delta^{15}N$ thresholds could be established to detect the application of inorganic fertilizers, forbidden in organic grape growing, as already done with relative success in other crops (Camin et al. 2011; Laursen et al. 2013). Nevertheless, there is still too little information with this regard in grapevines, so further research needs to be performed in order to establish the potential interest of these measurements for terroir characterization and for other agronomic applications.

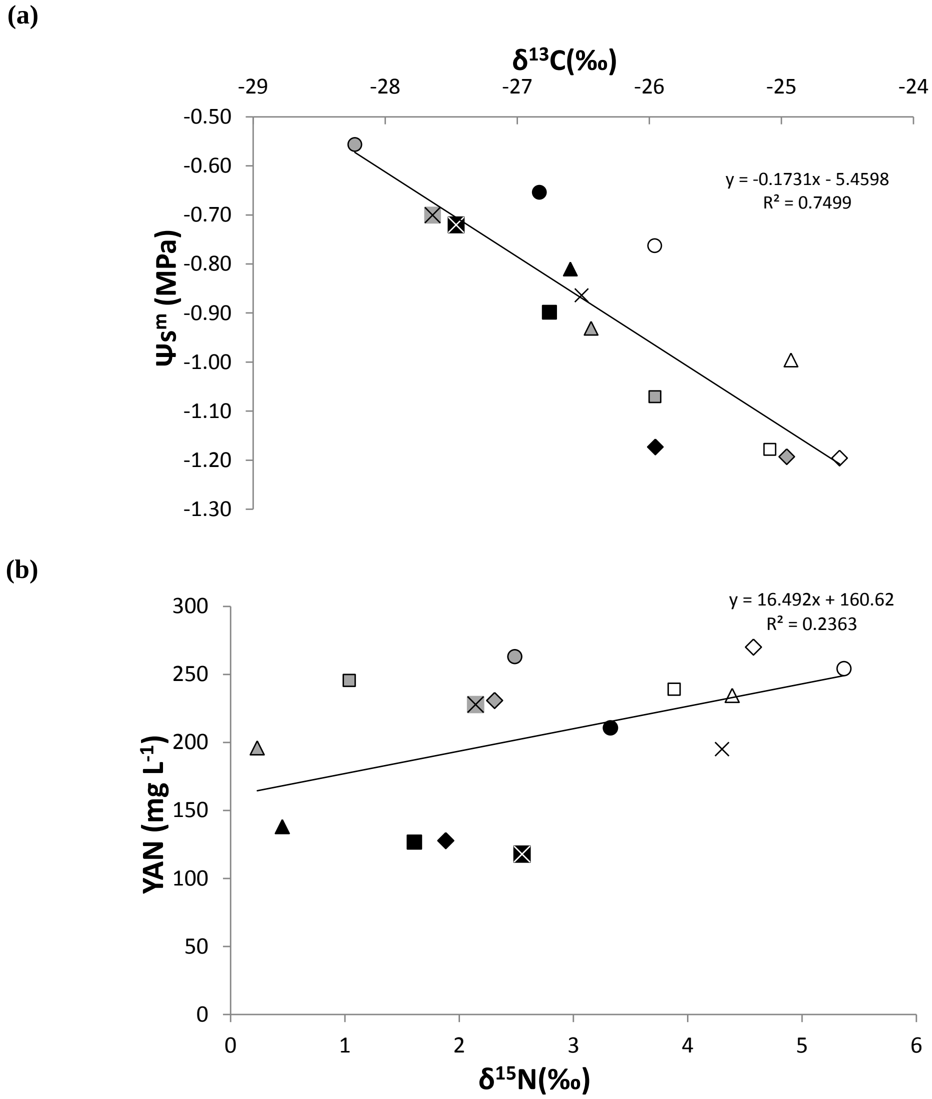

Figure 3: Relationship of (a) carbon isotope ratio ($\delta^{13}C$) of berries at harvest with mid-morning stem water potential (Ψs-m) between veraison and harvest, and of (b) nitrogen isotope ratio ($\delta^{15}N$) and yeast available nitrogen (YAN) of berries at harvest. *Points have been double-coded depending on the year as diamonds (2009), squares (2010), triangles (2011), circles (2012) and crosses (2013), and on the vineyard from which data were obtained (white: vineyard #1; grey: vineyard #2; black: vineyard #3)*

4 CONCLUSION

The study of the natural abundance of carbon and nitrogen stable isotope can be a powerful tool for *terroir* characterization and study, since they act as integrators of some characteristics such as seasonal water dynamics and vine nitrogen sources intrinsically linked to *terroir*. Nevertheless, further research is required to better understand how environmental conditions and agricultural practices affect them.

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