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NITROGEN ISOTOPE RATIO ($\Delta^{15}N$) AS A TOOL TO TRACE THE MAJOR NITROGEN SOURCE IN VINEYARDS

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Abstract

Aim: to elucidate if it is possible to detect variations in the source of nitrogen (organic vs. inorganic) measuring nitrogen isotope ratio ($\delta^{15}N$) in berries and to examine the degree of variation occurring for this parameter naturally within a vineyard.

Methods and Results: two nitrogen fertilization strategies based on the use of organic and inorganic nitrogen sources were compared through four consecutive seasons in a vineyard, and berry $\delta^{15}N$ was measured at harvest. The source of nitrogen affected remarkably nitrogen isotope ratio, as samples from organically fertilized vines always showed higher $\delta^{15}N$ values. Additionally, variations in berry $\delta^{15}N$ were measured during two seasons in a 60-node sampling grid in a 4.2 ha vineyard, showing that a wide range of variation existed for $\delta^{15}N$ within the vineyard, and that its values followed a structured pattern that was in accordance with variations in altitude, being lower in the highest parts of the field.

Conclusions: the source of nitrogen (organic vs. inorganic) affects berry $\delta^{15}N$. Nevertheless, the degree of variation observed naturally within a single field is very relevant, and associated to variations in altitude.

Significance and Impact of the Study: this is the first study that, to our knowledge, demonstrates a direct relationship between nitrogen source and nitrogen isotope ratio in grapevines, and opens the door to its use in grapevine nutrition and terroir studies.

Keywords: Nitrogen, fertilization, organic, inorganic, *Vitis vinifera* L.

Introduction

Viticulture research has already considered stable isotopes as a valuable source of information, the main applications having been reviewed in Santesteban *et al.* (2015). The most frequently analysed variations in isotope composition are those in carbon, as they have been shown to be a reliable estimator of plant water status along the season *(Gaudillere et al., 2002; Herrero-Langreo et al., 2013; Santesteban et al., 2016; van Leeuwen et al.,* 2009). There is also a relatively high number of research works dealing with hydrogen and/or oxygen isotopes, which mainly provide information on the water sources and on evaporation processes (some examples in Ingraham *et al.,* 1999; Martin *et al.,* 2003; West *et al.,* 2007). Quite surprisingly, there is nearly no research dealing with variations in nitrogen isotope forms in viticulture, with only two references found in research databases (Santesteban *et al.,* 2014; Stamatiadis *et al.,* 2007). Those two works, in spite of not presenting a very detailed research, already showed the potential interest of measuring variation in nitrogen isotope forms in grapevine samples.

Nitrogen has two stable isotopes in nature, ^{14}N and ^{15}N, mostly found as the lightest isotopic form ^{14}N (99.634%), whereas the heaviest form ^{15}N represents 0.366% of the total (Hoefs, 2009). As for most isotopes, the differences between samples in their nitrogen isotope composition are very small, and therefore they need to be represented as a ratio to the composition of an internationally accepted standard. This ratio is defined as the nitrogen isotope ratio ($\delta^{15}N$), calculated as detailed in Eq. 1, and the standard used as reference is atmospheric N_2 gas.

$$\delta^{15}N(‰) = \left(\frac{^{15}N_{sample}/^{14}N_{sample}}{^{15}N_{standard}/^{14}N_{standard}} - 1\right) \times 1000 \qquad \text{[Equation 1]}$$

Plant uptake of nitrogen throught the roots is known not to induce significant isotope discrimination during the absorption process, particularly when the external nutrient concentration is low (Santesteban et al., 2015) and references therein). On the contrary, there are substantial differences in the nitrogen isotope ratio ($\delta^{15}N$) between the different sources plants may take nitrogen from. In this regard, organic matter usually shows much higher $\delta^{15}N$ values than inorganic fertilisers (Bateman and Kelly, 2007). For example, ammonium nitrate fertilisers show a range of $\delta^{15}N$ between -1.4 and 2.6‰, whereas in manure and compost $\delta^{15}N$ ranges from 3.5 to 16.2‰, average values being 0.2 and 8.1‰, respectively. As a consequence, the source of N is the main factor determining the $\delta^{15}N$ values observed in plant tissues (Kendall *et al.,* 2007).

In this work, we present the results obtained in an experiment where two sources of nitrogen were applied (organic vs. inorganic), in order to elucidate if it is possible to trace those differences into the berries nitrogen isotope composition. Additionally, the degree of variation detected within one single field is also examined, in order to estimate the degree of naturally occurring variations associated to vineyard variability.

Materials and Methods

A field experiment was established in 2011 in a cv. Tempranillo vineyard in Traibuenas, Southern Navarre, Spain (42°22′N; 1°37′W; 340 masl), a region characterized by a semiarid climate (Bs type in Koppen's classification; $P < 350$ mm; ETPPenman > 1150 mm). The vineyard is trained as a vertical shoot positioned bilateral cordon, plant spacing was 3 (between rows) × 1 m (between plants in a row) and grafted to 110 Richter. The vineyard was 14 years-old at the beginning of the experiment, and bud load was fixed at 12 buds per m of row line.

Two nitrogen fertilization strategies were compared, based on the use of O (organic) and I (inorganic) nitrogen sources. In the case of O treatment, 5 t ha^{-1} of compost were incorporated into the alleys every January, whereas for treatment I, the amount of N and K equivalent to that provided by compost in organically fertilised vines was incorporated through two fertigation events, 2 weeks before and 2 weeks after budburst. N was added as ammonium nitrate. For all treatments, an additional basal application of inorganic N was done by the winery managers with a solid N-P-K fertilizer, equivalent 30 kg N ha^{-1} yr^{-1}.

For each treatment, eight replicates formed by five complete rows each were considered. All measurements and sampling were made in the central two rows, in 20 vines that were selected and marked at the beginning of the experiment based on their trunk cross-sectional area in order to reduce variability.

At harvest time, cluster number and yield were determined, and two berry samples taken from each replicate. One of the samples was used to determine total soluble solids, pH, titratable acidity (TA) and yeast assimilable nitrogen (YAN), whereas the second one was used to determine $\delta^{15}N$ in berries.

Additionally, and in order to explore the degree of variation that may exist within a vineyard due to differences in topography, $\delta^{15}N$ was analysed in 2010 and 2011 in berry samples that had been collected from the nodes of a grid of 60 sampling points (SP) in a rainfed vineyard in Leza (Basque Country, Spain). This field was 4.2 ha gobelet-trained cv. 'Tempranillo' vineyard. Planting frame was 2.4 m x 1.2 m, and the vineyard was 17-years old at the beginning of the experiment. Further details about this vineyard can be found in Urretavizcaya *et al.* (2014, 2017), where a complete agronomic characterization of the sampling gird is also provided. Within field variations in $\delta^{15}N$ field distribution were examined making maps through kriging analysis, and compared to elevation maps obtained from the Digital Elevation Model repository of the Spanish National Centre of Geographic Information (www.ign.es).

In both vineyards, samples were oven dried at 75 ºC and ground to a fine powder to determine $\delta^{15}N$. From each ground sample, three 2 mg subsamples were analysed for $\delta^{15}N$ using an Elemental analyser (NC2500, Carlo Erba Reagents, Rodano, Italy) coupled to Isotope Mass Spectrometer (Thermoquest Delta Plus, ThermoFinnigan, Bremen, Germany).

Results and Discussion

The source of N affected remarkably $\delta^{15}N$, samples from organically fertilized vines always showing higher $\delta^{15}N$ values in the four seasons, these differences being statistically significant from the second season on (Figure 1). The results obtained agree with those observed for other species, as there is no relevant isotope discrimination for N in the plants and, therefore, the $\delta^{15}N$ of plant tissues depends majorly on the $\delta^{15}N$ of the nitrogen source. In our case, $\delta^{15}N$ of the compost used ranged between 7.5 and 9.1 ‰ depending on the year, whereas that of the inorganic fertilizer ranged from -0.8 to -0.2 ‰.

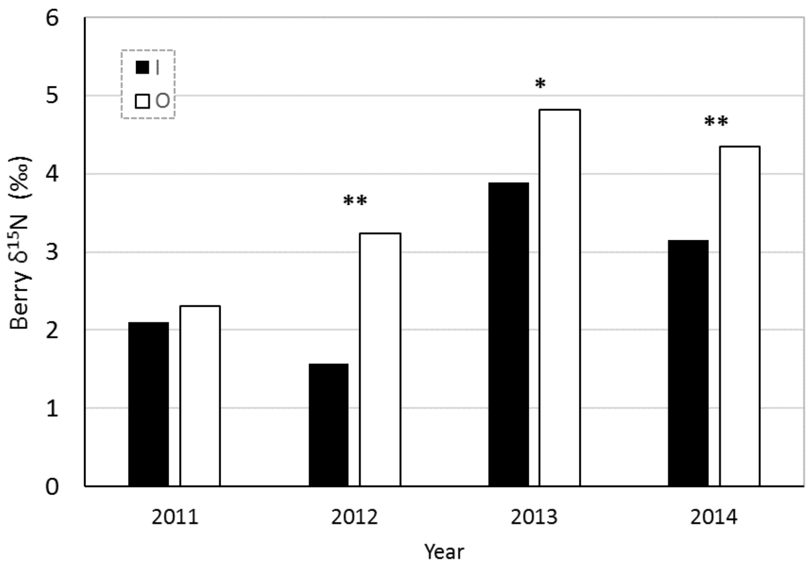

Figure 1: Effect of the source of nitrogen (O: organic, I: inorganic) on nitrogen isotope ratio ($\delta^{15}N$).

Regarding within field variability, the results obtained show that there is a remarkable degree of variation in $\delta^{15}N$ within the Leza vineyard, ranging from -2.9‰ to 9.6‰ (Figure 2, average data of 2010 and 2011 displayed). This range of variation is very relevant, though just slightly similar to those observed by Stamatiadis et al. (2007), who reported $\delta^{15}N$ values between 0.43‰ to 9.12‰. Nitrogen isotope ratio followed a structured pattern (i.e., values are not randomly distributed), that was very similar both years (maps not presented). When the observed patterns were compared to the elevation maps, a clear correspondence could be found (Figure 2), as $\delta^{15}N$ tended to be lower in those parts of the field at higher altitudes, and vice versa.

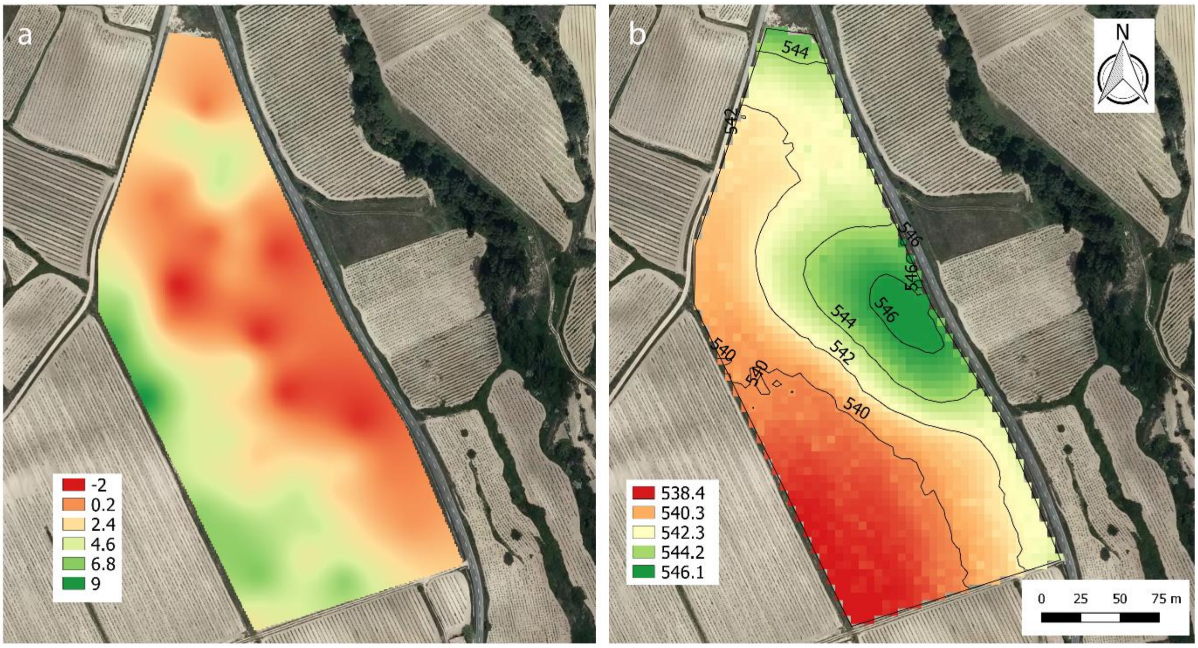

Figure 2: Map displaying spatial variations in (a) $\delta^{15}N$ in whole berries (average of 2010 and 2011) and (b) altitude in the vineyard in Leza.

Although we lack a detailed mapping of soil properties to provide a sound explanation for this trend, it can be hypothesised that the lowest areas have soils with higher contents in organic matter and, therefore, vines in those areas can rely more on nitrogen of organic origin to meet their demand. This trend agrees with that observed by Stamatiadis *et al.* (2007) in one of the two vineyards they included in their research in Greece, where leaf δ^{15} N values were lower in the upland positions. Nevertheless, these authors found an opposite trend in the other field they mapped for this variable, showing that the interpretation of spatial differences in $\delta^{15}N$ may be more complex than those related to $\delta^{13}C$ described in earlier research works (Herrero-Langreo *et al.,* 2013; Santesteban *et al.,* 2017; van Leeuwen *et al.,* 2018).

Conclusions

These results are, to the best of our knowledge, the first to establish a link between the source of nitrogen and $\delta^{15}N$ composition in grapevine berries, proving that this information can be used as an indicator of the nitrogen source. Nevertheless, our results also prove that there is a large degree of variation in $\delta^{15}N$ within a field, which needs to be taken into account for the interpretation of this analysis. Further research on other potential sources of variability in $\delta^{15}N$ and on suitability of different vine organs to measure $\delta^{15}N$ is required, as well as analyzing.

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