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ORIGINAL RESEARCH ARTICLE

Nitrogen isotope ratio ($\delta^{15}N$): a nearly unexplored indicator that provides useful information in viticulture

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ABSTRACT

The study of the natural abundance of nitrogen-stable isotopes is an aspect of viticulture research that has received limited attention. While stable isotopes of carbon, oxygen and hydrogen have received significant attention, nitrogen isotope ratio ($\delta^{15}N$) remains a less studied yet potentially informative parameter. This paper explores the implications of $\delta^{15}N$ measurements in grapevines, shedding light on its utility as an indicator for nitrogen sources, plant water status, and within-field variability. The study examines the influence of nitrogen sources, dose, and sampling organs on $\delta^{15}N$ values, revealing distinct trends in petioles, berries, and seeds. Organic fertilisers led to higher $\delta^{15}N$ values compared to inorganic sources, while increasing nitrogen doses exhibited a much weaker effect on $\delta^{15}N$. Moreover, $\delta^{15}N$ values vary spatially within vineyards, associating with its topography and potential soil composition, soil management and water availability. Our results highlight the importance of considering $\delta^{15}N$ in viticulture, suggesting its integration with $\delta^{13}C$ for comprehensive insights into nitrogen cycling and soil management practices. The findings advocate for further research to harness the full potential of $\delta^{15}N$ as a valuable tool in viticultural studies.

KEYWORDS: Nitrogen isotopes, $\delta^{15}N$, nitrogen sources, plant water status, within-field variability

INTRODUCTION

1. Nitrogen isotope variation studies in viticulture

Isotopes are defined as species of atoms of a chemical element located in the same position in the periodic table. Thus, isotopes share an atomic number, i.e., they have the same number of protons but have different atomic mass and physical properties, as the number of neutrons in their nuclei is different. They can be classified into two major groups: stable (maintaining a constant concentration on Earth over time) and radioactive (disintegrating at predictable rates to form other isotopes).

For most light elements, such as hydrogen, carbon, nitrogen and oxygen, one of the isotopes is greatly prevalent (>98 % of the atoms belong to that stable isotope form), the others being present only in trace amounts. The concentration of isotopes in natural compounds varies due to their slightly different mass-dependent behaviour in natural processes. As isotope discrimination is a function of the parameters characterising the process, the study of the relative content of other stable isotope forms can be very informative for many disciplines, ranging from human nutrition (Davies, 2020) to paleobiology (Fisher, 2018) and planetary sciences (Joy *et al*., 2020). In the particular case of plant and environmental sciences, the stable isotopes most frequently considered are those included in the four light elements mentioned above (Marshall *et al*., 2007), although there are also interesting applications for the study of the stable isotopes of other elements such as B, Ba, Ca, S or Sr (Bullen and Chadwick, 2016; Dawson *et al*., 2002; Sun *et al*., 2018). The relevance of these forms is due to their abundance on the Earth's surface and their involvement in relevant biological processes (Adams and Grierson, 2001). The stable isotopes of heavier elements, such as B, S, Sr and Mg, are also used in plant research, though less often.

Viticulture has already considered stable isotopes a valuable source of information, the main applications of which have been reviewed by 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 amount of research dealing with hydrogen and/or oxygen isotopes, which mainly provide information on the water sources and evaporation processes (Ingraham and Caldwell, 1999; Martin and Martin, 2003; West *et al*., 2007).

Studying nitrogen-stable isotopes in plant and environmental sciences is a useful tool to better understand N cycling processes and provide insights into historical N availability and ecosystem dynamics (Craine *et al*., 2015). In viticulture, although ^{15}N measurement has been used quite profusely when artificially enriched sources of N are added to study the dynamics of N absorption and translocation (Baldi *et al*., 2017; Hajrasuliha *et al*., 1998; Morinaga *et al*., 2003; Schreiber *et al*., 2002; Verdenal *et al*., 2021; Vos *et al*., 2004; Walker *et al*., 2022; Zapata *et al*., 2004), or even in paleobotanical research (Joka *et al*., 2024), quite surprisingly, it has not been until the last years that research in viticulture has incorporated the measurement of the natural abundance of nitrogen isotope forms (Santesteban *et al*., 2014; Stamatiadis *et al*., 2007). Grapevine leaf, cane and must samples show lower $\delta^{15}N$ values than those of the corresponding soil (Durante *et al*., 2016; Paolini *et al*., 2016), as the bulk $\delta^{15}N$ of plant tissue depends not only on inorganic primary nitrogen sources but also on isotope fractionation during uptake and assimilation. More recently, works conducted in Switzerland have investigated the impact of soil management and water availability on $\delta^{15}N$ in solid wine residues, observing decreased $\delta^{15}N$ values associated with water stress and the competitive effect of a cover crop (Spangenberg and Zufferey, 2018; Spangenberg and Zufferey, 2023), and the same team has lately investigated variations in $\delta^{15}N$ grapevine leaves as affected by early water stress and leaf age (Spangenberg *et al*., 2020; Spangenberg *et al*., 2021). Altogether, the works presented above constitute only a small set of information but show the potential interest of measuring the variations in nitrogen isotope forms that occur naturally in grapevine.

Similarly, it is remarkable that while within-field variations in carbon isotope ratio ($\delta^{13}C$) have been quite frequently reported (Herrero-Langreo *et al*., 2013; Santesteban *et al*., 2017; Van Leeuwen *et al*., 2018), there is only one work that, to our knowledge, has reported variations of $\delta^{15}N$ at a within-field scale (Stamatiadis *et al*., 2007). Within-field variability is a feature that it increasingly considered in vineyard management due to the development of precision agriculture (Santesteban *et al*., 2019). Many studies consider the implications of variations between parts of a vineyard on agronomic performance (Bramley *et al*., 2019; Ledderhof *et al*., 2017; Urretavizcaya *et al*., 2017; Verdugo-Vásquez *et al*., 2018), those variations being mainly related to variations in soil composition and depth associated with changes in topography (Bramley *et al*., 2011; Santesteban *et al*., 2013; Scarlett *et al*., 2014). However, there is much less information on how changes in soil characteristics may affect nutrition and how these variations should be considered to implement variable rate fertiliser application strategies (Gatti *et al*., 2018; Gatti *et al*., 2019), and the study of $\delta^{15}N$ could be relevant in this regard.

2. Origin of the natural variations in nitrogen isotopes

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). Variations in nitrogen isotope compositions are measured as the relative deviation of the sample heavy-to-light isotope ratio $^{15}N/^{14}N$ from the international reference, that is, atmospheric N_2 gas, i.e., the nitrogen isotope ratio ($\delta^{15}N$), calculated as detailed in Eq. 1, and expressed either as its *per mille* (‰) value or as mUr (1 mUr = 1 ‰)

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

Plant uptake of nitrogen through the roots is known not to induce significant isotope discrimination during the absorption process, particularly when the external nutrient concentration is low (Billy *et al*., 2010; Santesteban *et al*., 2015). On the contrary, there are substantial differences in the nitrogen isotope ratio ($\delta^{15}N$) among the 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 ‰, while in manure and compost, $\delta^{15}N$ ranges from 3.5 to 16.2 ‰, the average values being +0.2 and +8.1 ‰, respectively. The typical range for $\delta^{15}N$ values in plant tissues is around –10 ‰ to +10 ‰ (Craine *et al*., 2015), and the source of N is the main factor determining the $\delta^{15}N$ values observed in plant tissues (Kendall *et al*., 2007). Some environmental factors, such as water availability and temperature, influence N mineralisation, NH_3 volatilisation, and denitrification processes and may, therefore, change the $\delta^{15}N$ of the source N in soil solutions (Högberg, 1997). In this regard, denitrification is known to induce ^{15}N enrichment of the residual nitrate (enrichment factor between −15 and 30 ‰), volatilisation and nitrification also cause isotopic depletion (average enrichment factors −20 ‰ and −25 ‰, respectively), whereas ammonification usually causes only a small fractionation (−1 ‰) (Billy *et al*., 2010; Kendall *et al*., 2007). Additionally, ^{15}N to ^{14}N fractionation occurs during uptake, translocation and assimilation can also affect $\delta^{15}N$ (Kalcsits *et al*., 2014), the latter contributing to a greater extent to the changes observed (Craine *et al*., 2015; Evans, 2001). as the enzymatic reactions involved selectively generally favour lighter isotopes (^{14}N) over heavier isotopes (^{15}N). Furthermore, for a certain plant organ, the relative contributions of newly absorbed N and remobilised N from different plant reserve organs can modify its $\delta^{15}N$ (Kolb and Evans, 2002; Robinson *et al*., 2000; Spangenberg *et al*., 2021).

Taking all the previous into account, there is a need for generating knowledge that permits understanding sources of $\delta^{15}N$ variation in grapevines. In this work, we present the results of several independent experiments in an attempt to highlight the potential interest of using this measure in viticulture.

MATERIALS AND METHODS

1. Experimental designs

1.1. Influence of the source of nitrogen on $\delta^{15}N$

1.1.1. Comparison of organic vs. inorganic nitrogen

As outlined in the introduction, according to the literature of research performed in other crops, the source of N is the main factor determining the $\delta^{15}N$ values observed in plant tissues (Kendall *et al*., 2007). To our knowledge, no experiment has evaluated this effect in grapevines under field or pot conditions. To determine the influence of nitrogen source on tissue $\delta^{15}N$ in vines, a field experiment was established at a cv. Tempranillo vineyard in Traibuenas (Navarra, northern Spain). Vineyard characteristics are summarised in Table S1.

The experiment started in 2011, was maintained for four consecutive seasons, and consisted of two treatments, labelled as O (organic) and I (inorganic), which differed in the major source of nitrogen used for fertilisation. In the case of O, five t ha^{-1} of compost were incorporated into the alleys every January, whereas, for I, the equivalent amount of the N and K that compost added was incorporated through two fertigation events, two weeks before and two weeks after budburst, when N was added as ammonium nitrate. Table S2 includes the characteristics of the composts used each season. For all treatments, an additional base application of inorganic N was performed with a solid N–P–K fertiliser, equivalent to 30 kg ha^{-1} yr^{-1} N.

For each treatment, eight replicates formed by five complete rows 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 to reduce variability.

1.1.2. Influence of the dose of inorganic nitrogen

To discern if the amount of nitrogen applied could affect $\delta^{15}N$, a similar experiment was set up in a vineyard adjacent to that described in the previous subsection, its characteristics being summarised in Table S1. The experiment was carried out along four consecutive seasons (2011–2014) and included four treatments that consisted of the application of 0, 50, 100 and 200 kg of N ha^{-1} each season (named N0, N50, N100 and N200). For each treatment, five replicates of 40 consecutive vines were included, received different doses of nitrogen during four consecutive seasons, and measurements were made in 10 vines from each replicate that were selected and marked at the beginning of the experiment based on their trunk cross-sectional area.

1.2. Influence of the organ sampled on $\delta^{15}N$

Different vine organs have been used as the source of information in vineyards, and all the data on petioles, whole berries, and seeds was pooled along four consecutive seasons in the experiments detailed in the previous subsection.

To determine which sampling organ could be more suitable under experimental conditions, we used the complete data set from Experiments 1 and 2 to calculate the Discrimination Ratio (DR) for each organ. This approach has already been used successfully to compare the discriminating ability of water potential measurements in grapevines (Cole and Pagay, 2015; Santesteban *et al*., 2011, 2019) and follows the principles described in Levy *et al*. (1999) and Browning *et al*. (2004), that compare the variability observed within samples of the same treatment and the underlying variability between treatments. Briefly, the intrinsic (within) variability of each organ is the mean standard deviation (SD) of the measurements obtained from the different replicates (SD_w) for each treatment, experiment and year. Then, the extrinsic (between) variability was estimated through the calculation SD of the mean values measured of the different treatments (SD_b) in each experiment and year and was corrected using SD_w to estimate the underlying SD (SD_u) as indicated in Eq. 2, where SD_u represents an unbiased estimate of the SD and k accounts for the number of replicates available. Finally, the DR was calculated as indicated in Eq. 3, and the DR was calculated for each organ compared by pairwise t-tests.

$$SD_u = \sqrt{SD_b^2 + \frac{SD_w^2}{k}} \qquad \text{[Eq. 2]}$$

$$DR = \frac{SD_u}{SD_w} \qquad \text{[Eq. 3]}$$

Additionally, to compare the possible interest of dormant canes as a source of integrative information, two cv. Tempranillo vineyards on the same farm were selected in 2020, and three replicated field samples were collected at harvest for berries and in winter for the basal, mid, and upper parts of dormant canes.

1.3. Within vineyard variations in $\delta^{15}N$

To explore variations in nitrogen isotope ratio, samples obtained in two precision viticulture experiments performed by our team were analysed to determine $\delta^{15}N$. The data presented correspond to a cv. Tempranillo dry-farmed vineyard located in Leza (Basque Country, northern Spain) and to an irrigated cv. Tempranillo field in Traibuenas (Navarra, northern Spain). Field data were taken in the 2010 and 2011, as well as the 2015 and 2016 seasons. Vineyard characteristics are summarised in Table S1, and all details on the experiment layout are detailed, respectively, in Urretavizcaya *et al*. (2013) and Matese *et al*. (2019). Briefly, a grid of sampling points (SP) was established in each vineyard (60 SP in Leza and 92 in Traibuenas) following a square regular grid (30 m × 30 m in Leza, 25 × 25 m in Traibuenas). Each SP was made up of 10 vines located in two adjacent rows. Information on the altitude of the vineyards was extracted from the Digital Elevation Model repository of the Spanish National Center of Geographic Information (www.ign.es).

1. Plant measurements

In all experiments, agronomic evaluation was conducted following standard procedures. In short, as agronomic features, yield and its components were determined by counting and weighing all clusters produced in ten vines per replicate or sampling point. Berry composition was determined using two berry samples per replicate or sampling point. Samples were carried to the lab at low temperature (4–6 °C) for analysis, weighed to determine mean berry weight (BW), and a 100-berry subsample homogenised with an LMU 9018 American blender (Man, México) for 10 s at full speed. Part of this homogenate (100 g approx.) was filtered with a gauze tissue and used to measure total soluble solids (TSS) and pH. Yeast assimilable nitrogen (YAN) was determined using Fourier-transform infrared spectroscopy (FTIR), and total anthocyanins and phenolics were measured following the Cromoenos® method using 200-berry subsamples. This method consists of a fast extraction of phenolics following a procedure and reagents provided by the Bioenos company (www.bioenos.com) and has been shown to predict wine colour and composition similarly or even better than other classical procedures (Kontoudakis *et al*., 2010).

In terms of the sampling used to determine nitrogen isotope composition, the same structure in the experiments designed to determine the influence of the source of nitrogen and the amount of inorganic nitrogen added was used (experiments 1.1. and 1.2. in methodology). At veraison, a 25-petiole sample was taken at each replicate to determine the N content and $\delta^{15}N$ and, at harvest, two 50-berry samples per replicate were taken, one being used to determine $\delta^{15}N$ in whole berries and the other to determine $\delta^{15}N$ in seeds. In all cases, samples were oven-dried at 75 °C and ground to a fine powder prior to $\delta^{15}N$ analysis. In the analyses performed to evaluate within-field variability (experiment described in point 1.3), 50 berry samples were taken at harvest from each SP. In the case of the vineyard in Leza, samples were oven-dried, ground to a fine powder, and then analysed, while those from Traibuenas vineyard were analysed using filtered and oven-dried must samples.

Carbon and nitrogen isotope ratio determinations were carried out using, for each biological replicate, three 2 mg technical subreplicates, using an Elemental analyser (NC2500, Carlo Erba, Reagents, Rodano, Italy) coupled to an Isotope Mass Spectrometer (Thermoquest Delta Plus, ThermoFinnigan, Bremen, Germany). Must samples were packed in tin capsules for conversion into CO_2 and N_2 in an elemental analyser (Carlo Erba CHNSO 1108) coupled to an isotope ratio mass spectrometer (Finnigan Mat Delta Plus). Both C and N isotope composition is reported in the delta (δ) notation, the standards being, respectively, the Vienna Peedee Belemnite (V-PDB) and the molecular nitrogen in air (Air-N_2).

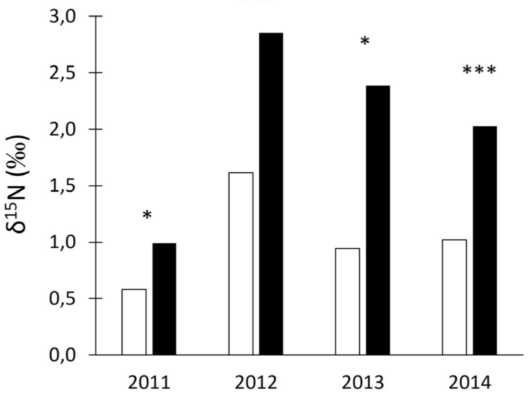
2. Data analysis

The statistical analysis to assess the differences among the treatments was carried out using one-way analysis of variance (ANOVA). Upon establishing the statistical significance of the overall ANOVA, when appropriate, Duncan's post hoc test was conducted at $P < 0.05$ to identify specific pairwise differences between treatment groups and the assumptions of ANOVA, including normality and homogeneity of variances, were assessed. Linear regression analysis was employed to assess the relationship between variables. Statistical analyses were performed using R statistical software (R Core Team, 2022). Spatial variability of isotope ratios was assessed using kriging, a geostatistical technique that interpolates and predicts values at unsampled locations based on the spatial autocorrelation of the observed data. The kriged maps were generated using QGIS software v.3.16.

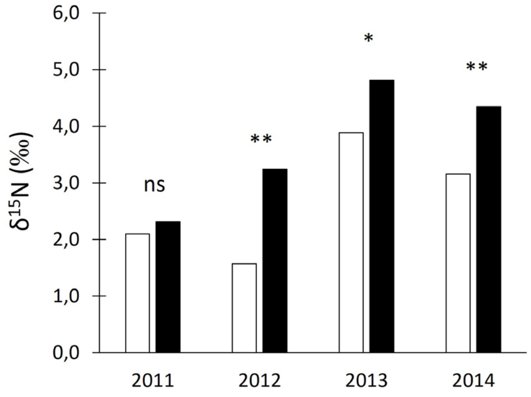
RESULTS AND DISCUSSION

The results of the agronomic performance of the vineyards considered in this research are presented as supplementary material (Tables S3, S4 and S5). This information, although not central in this article discussion, can be useful to

(a) Petioles



(b) Berries



(c) Seeds

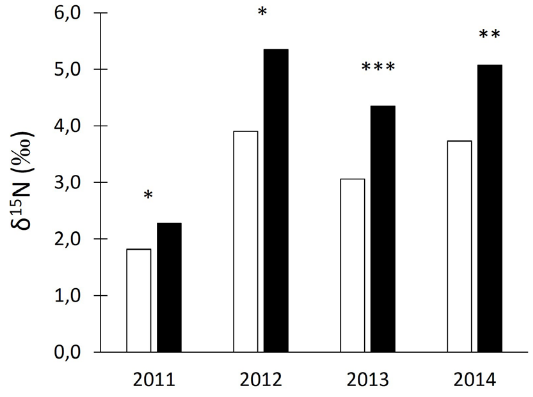


FIGURE 1. Effect of the source of nitrogen on δ15N content in (a) petioles, (b) whole berries and (c) seeds. White and black columns correspond, respectively, to inorganic and organic sources.

ns: not significant differences (P > 0.05); *, **, ***: significant differences with P-values < 0.05, < 0.01 and < 0.001, respectively.

contextualise the results obtained and, therefore, is made available.

1. Influence of the source of nitrogen on δ15N

The source of N affected the $\delta^{15}N$ content in the three organs considered (Figure 1), with samples from organically fertilised vines showing higher $\delta^{15}N$ values in the four seasons. Differences were observed in petioles and seeds during the four years, whereas in whole berries, they were observed from the second season on. The results obtained agree with those observed for other species (Bateman and Kelly, 2007; Camin *et al*., 2011; Choi *et al*., 2002, 2017; Mie *et al*., 2022) since the main driver of $\delta^{15}N$ is the nitrogen source due to the relatively lower magnitude of isotope discrimination for during absorption and assimilation of N (Durante *et al*., 2016; Paolini *et al*., 2016; Santesteban *et al*., 2015). 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 fertiliser ranged from –0.8 to –0.2 ‰.

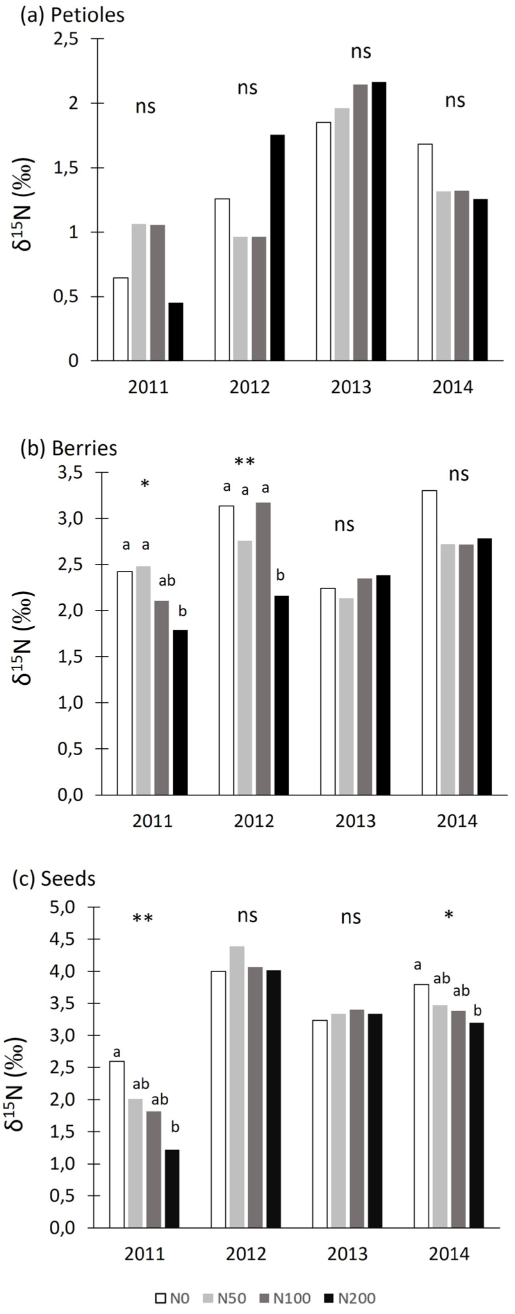


FIGURE 2. Effect of the dose of inorganic nitrogen in the δ^{15}N in (a) petioles, (b) whole berries and (c) seeds.

ns: not significant differences ($P > 0.05$); *, **, ***: significant differences with P-values < 0.05, < 0.01 and < 0.001, respectively. Columns with different letters correspond to groups defined according to Duncan's post hoc test.

The effect of the amount of inorganic nitrogen on δ^{15}N was much smaller than that observed with compost application and led to a slight decrease in δ^{15}N for those treatments where the N doses were higher during the first years of the experiment (Figure 2). The increasing amount of inorganic nitrogen available probably made plants less dependent on nitrogen organic sources, resulting, therefore, in lower δ^{15}N values. Although the incidence of the amount of nitrogen added has been slight, it needs to be considered that, due to the low organic matter content of the soil of this vineyard (1 %), the amount of nitrogen of organic origin that may be available is really low. Nevertheless, in vineyards where soil organic matter content is higher, the impact of the addition of inorganic nitrogen on δ^{15}N could be more relevant, as observed by Liu *et al*. (2013) in forest species.

These results are, to the best of our knowledge, the first to establish a link between the modification of the potential sources of nitrogen and δ^{15}N in grapevines and show that all the organs considered (petioles, whole berries, and seeds) can be used as sensitive indicators of the nitrogen source. The impact of different doses of inorganic was also detected,

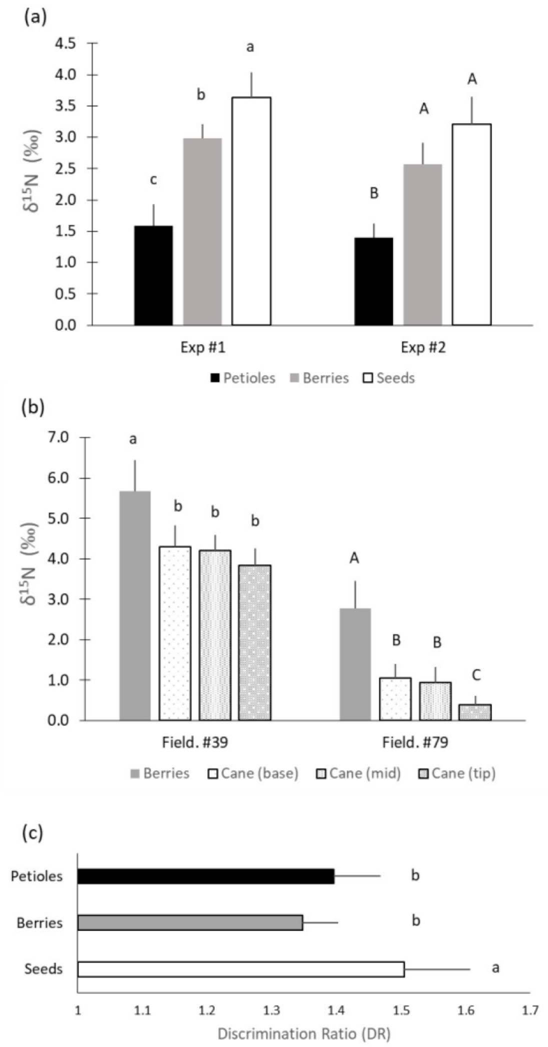


FIGURE 3. Comparison of (a) $\delta^{15}N$ in petioles, whole berries and seeds, (b) $\delta^{15}N$ in berries and basal, mid and upper sections of dormant shoots and (c) of the Discrimination Ratio of petioles, whole berries and seeds.

The bars indicate standard error, and letters correspond to different groups as calculated with t-tests. Each experiment was evaluated separately, as denoted by differences in letter capitalisation.

although, under these experimental conditions, variations were much smaller.

2. Influence of the organ measured

Nitrogen isotope ratios showed a consistent trend to be lower in petioles, followed by whole berries, the highest values being observed in seeds (Figure 3a). Similarly, when the $\delta^{15}N$ values observed in dormant shoots were compared to those in berries in samples from two cv. Tempranillo fields located on the same farm, there is also a trend toward lower $\delta^{15}N$ in the vegetative organs (Figure 3b) than in the berries. This trend to observe higher $\delta^{15}N$ in fruits agrees with the observations of Pascual *et al*. (2013), who showed that fruit $\delta^{15}N$ were ≈2 ‰ higher than in leaves. On the contrary, in rice, differences between organs were much smaller, and leaves exhibited higher $\delta^{15}N$ values than grains, stems, and roots (Wang *et al*., 2022). Within the cane, we observed a slight trend to have higher $\delta^{15}N$ values at the basal and mid sections than at the upper section (Figure 3b), this trend not being coincident with the observations of Spangenberg *et al*. (2021) for leaves sampled at those positions in the shoot.

The DR values obtained to compare the suitability of petioles, whole berries and seeds (Figure 3c) as the source of information on the source of the nitrogen used by the vines show that seeds were the most informative organ. The higher DR values indicate that seeds can significantly discriminate better between treatments. Seed nitrogen concentration is known to be greater than that in pulp (Bell and Henschke, 2005), and it could be hypothesised to be, as a consequence, more sensitive to changes in the nitrogen source. However, this statement needs to be confirmed under other experimental conditions that can certainly affect this behaviour. In any case, it is necessary to highlight that, provided the differences in

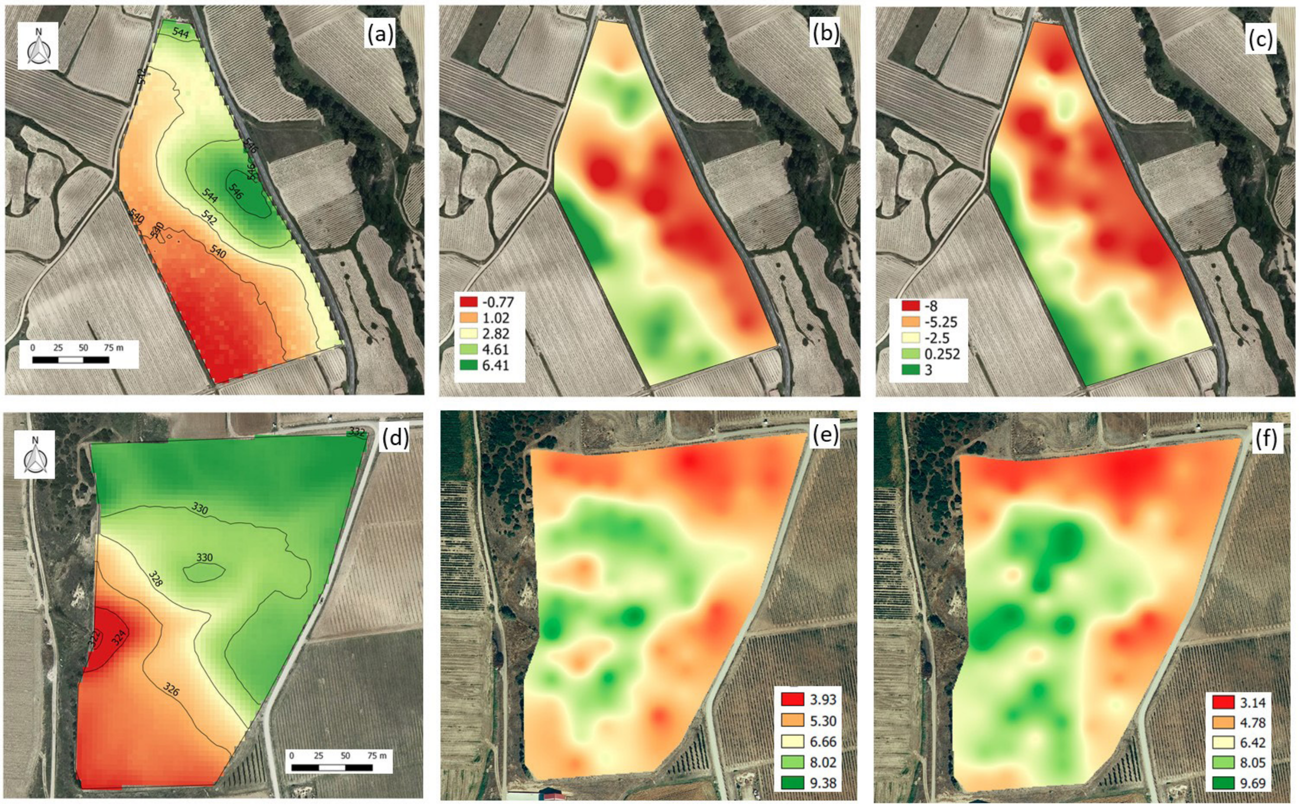


FIGURE 4. Within field variability of altitude and nitrogen isotope ratio in berries sampled in Leza (a) altitude, (b) δ^{15}N (‰) in 2010, (c) δ^{15}N (‰) in 2011; and Trabuenas (d) altitude, (e) δ^{15}N (‰) in 2014, (f) δ^{15}N (‰) in 2015.

δ^{15}N among organs are a consequence of a complex interplay between uptake, losses, assimilation and translocation of nitrogen, their comparison could be used as an integrated metric for understanding better nitrogen fluxes, assimilation processes, and allocation dynamics within plant systems (Cui *et al*., 2020; Kalcsits *et al*., 2014).

3. Variations within field level

The results obtained show that there is a noticeable degree of variation in δ^{15}N within a single field, this degree of variation ranging from - 8.0 ‰ to 6.4 ‰ in Leza and from 3.1 ‰ to 9.7 ‰ in Traibuenas. This range of variation is very relevant, similar to those reported by Stamatiadis *et al*. (2007), who reported δ^{15}N values between 0.43 ‰ to 9.12 ‰ within a vineyard in Greece.

The nitrogen isotope ratio in both fields followed a structured pattern (i.e., the values are not randomly distributed), and the pattern observed in both years is stable, without notable changes from one year to another (Figure 4). When the observed patterns are compared to the elevation maps, a clear correspondence can be found, as δ^{15}N tended to be lower in those parts of the fields at higher altitudes and vice versa (Figure 4). As altitude is associated with soil properties such as texture, horizon depth and organic matter, the effect observed is probably an indirect consequence of these changes.

The trend observed agrees with that observed by Stamatiadis *et al*. (2007) in one of the two vineyards included in their research, where leaf δ^{15}N values were lower in the upland positions. However, these authors found an opposite trend in the other field they mapped for this variable, showing that the interpretation of spatial and temporal differences in δ^{15}N may be complex. Similarly, Santesteban *et al*. (2014), when comparing δ^{15}N values in berries sampled in three vineyards at a single location during five consecutive seasons, reported consistent differences between vineyards; the grave soil always resulting in the highest δ^{15}N values, probably as a consequence of increased N leakage in spring. The differences between years were less than those observed between vineyards and were attributed to differences in the soil mineralisation dynamics in spring.

To evaluate if variations could be indirectly associated with plant water status, as δ^{15}N has been observed to react to water status in Switzerland (Spangenberg and Zufferey, 2018), we compared δ^{15}N and δ^{13}C both seasons through regression analysis (Figure 5). The regression coefficients confirmed that there is a strong stability in δ^{15}N values between years ($R^2_{LEZA} = 0.68$, $R^2_{TRAIBUENAS} = 0.70$, Figure 5a), which was similarly observed for plant water status estimated with δ^{13}C ($R^2_{LEZA} = 0.64$, $R^2_{TRAIBUENAS} = 0.61$, Figure 5b). However, when isotope ratios are compared with each other, the correlation coefficients are very low, though statistically significant in two of the four vineyard-year combinations ($R^2_{LEZA\#1-} = 0.04$, $R^2_{LEZA\#2-} = 0.24$, $R^2_{TRAIBUENAS\#1} = 0.01$; $R^2_{TRAIBUENAS\#2} = 0.08$, Figure 5c,d). These relationships, although weak, occur in the same direction, the greater δ^{15}N

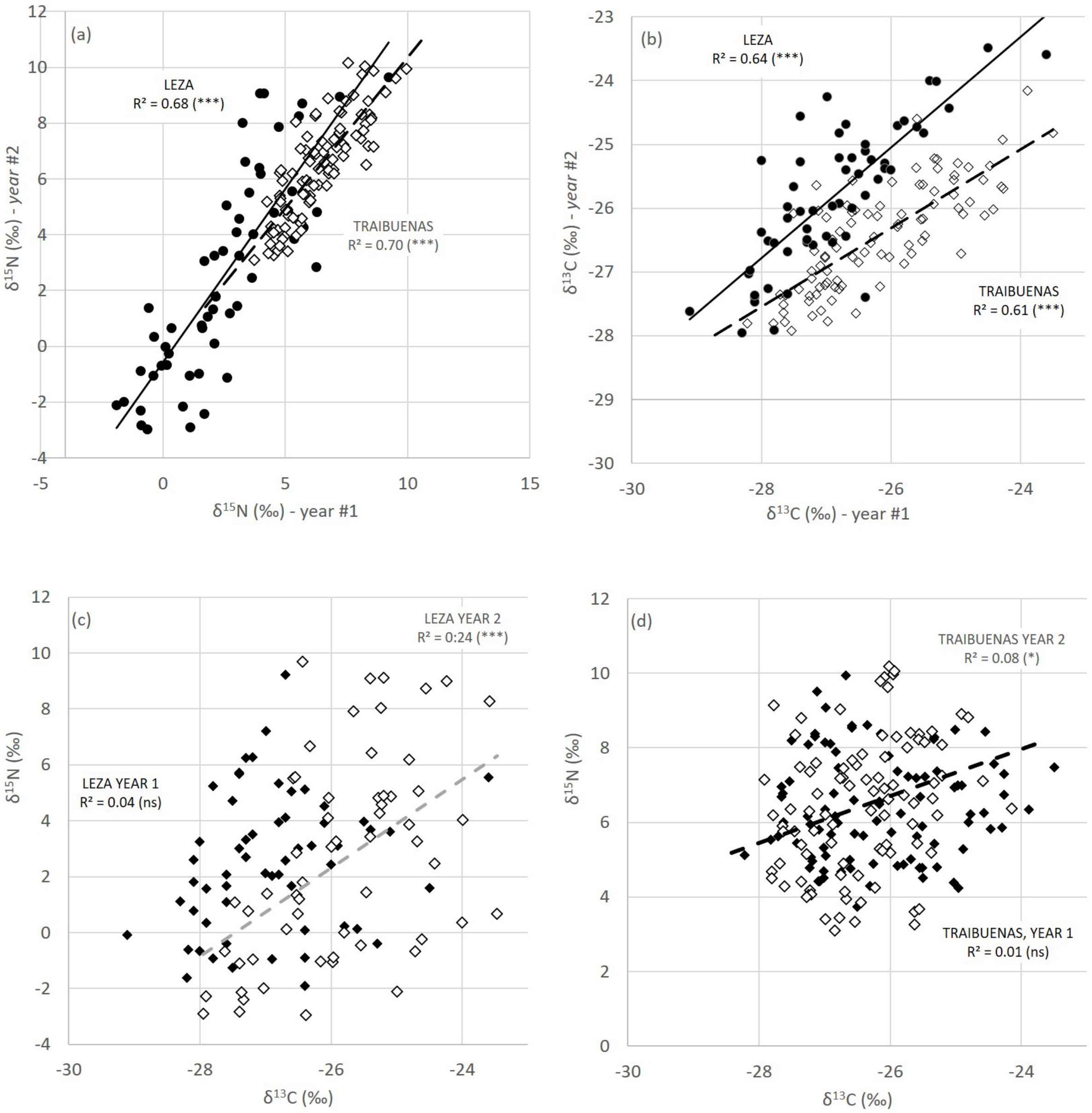


FIGURE 5. Comparison of (a) $\delta^{15}N$ values observed in berries at the sampling points in the two seasons within each vineyard, (b) $\delta^{13}C$ values observed at the sampling points in the two seasons within each vineyard, and of the values of $\delta^{15}N$ vs $\delta^{13}C$ each year in (c) Leza and (d) Traibuenas.

being associated with higher $\delta^{13}C$, i.e. to greater water stress conditions, the opposite reported in (Spangenberg *et al.*, 2020; Spangenberg and Zufferey, 2018) in Switzerland. However, it's important to note that the magnitude of water status variations in our study is moderate. In contrast, the aforementioned research induced differences in water status through differential irrigation. As a result, these findings should be interpreted cautiously.

4. Final remarks

The complexity of the sources of variation in the $\delta^{15}N$ of plant tissues and relationships makes clear that straightforward interpretations may not capture the full picture. However, incorporating this information into research in viticulture, especially eco-physiological and agronomic studies involving cover crops, varied fertilisation strategies, and different levels of water stress, could provide valuable insights. It is particularly relevant that, as suggested by Spangenberg and Zufferey (2023), one uses a dual isotope approach that considers and interrelates $\delta^{13}C$ and $\delta^{15}N$. The coupling of $\delta^{13}C$ and $\delta^{15}N$ would be useful in this context to understand soil organic matter sources and carbon and nitrogen cycling under various land management practices (Park *et al.*, 2023).

At this stage, more experiments are needed to fully understand the potential applications of $\delta^{15}N$ information in viticulture. Therefore, additional collaborative efforts are needed to build a comprehensive database on this parameter.

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