

Isotopes do distinguish production system in Brazilian viticulture

Letícia Leonardelli¹, Susiane Leonardelli² and Joséli Schwambach¹

¹ Universidade de Caxias do Sul (UCS) / Instituto de Biotecnologia. Rua Francisco Getúlio Vargas, 1130; 95070-560 Caxias do Sul, Rio Grande do Sul, Brasil.

² Laboratório de Referência Enológica Evanir da Silva (LAREN)/SEAPI. Avenida da Vindima, 1855; 95084-470 Caxias do Sul, RS, Brasil.

Abstract. Environmental concerns about the impact of conventional agriculture have increased the demand for sustainable farming and reduced synthetic fertilizers, especially because unused nitrogen can lead to pollution. This study investigated the effect of organic and synthetic fertilizers on carbon and nitrogen isotopes in red and white grape cultivars to differentiate between organic, biodynamic and conventional production systems. Considering red and white cultivars, 120 grape samples were analysed, consisting of 60 organic and 60 conventional. The results showed that nitrogen values were significantly higher in organic grapes than in conventional, with no differences between red and white varieties within the same fertilization group. Additionally, no significant differences were observed between organic and biodynamic grapes. For carbon, significant differences were observed between organic and biodynamic systems compared to conventional for red grapes, while no significant differences were found for white grapes. The differences found may be attributed to the influence of nitrogen fertilization on photosynthesis and water use efficiency. The study demonstrates that nitrogen isotope composition is a valuable tool for identifying production systems. At the same time, carbon can complement nitrogen results, offering a promising approach for assessing and verifying grape cultivation practices.

1. Introduction

Environmental concerns related to conventional agriculture's impact, such as soil fertility decline, water scarcity, loss of biodiversity, and increasing herbicide resistance, have intensified the demand for more sustainable farming practices. This has led to a growing search for environmentally conscious and less invasive initiatives that promote the reduction of synthetic fertilizers and pesticide use [1, 2, 3].

Over the past few decades, organic viticulture has been expanded, driven by consumer demand for healthier food and environmentally friendly farming practices [4]. The transition of vineyards to organic production has accelerated significantly in the last years. In 2018, organic viticulture accounted for 422,300 ha worldwide [5]. A report by the International Organisation of Vine and Wine (OIV) indicates an annual increase of 13% since 2005 [6]. Brazil is following this global trend in organic agriculture, showing a substantial progress in recent years [7]. Rio Grande do Sul is the largest producer of organic grapes in

Brazil and has seen an increase of approximately 100% in organic wine production between 2019 and 2023, and 34% in organic juice between 2020 and 2023 [8].

Biodynamic agriculture is similar to organic farming, with a few additional principles, where the organism is regularly managed through the use of internal resources and specific preparations [9]. In this practice, alongside compost fertilization and cover cropping specific fermented plant preparations are used to enhance soil fertility and increase microbial diversity [10].

Nitrogen isotopic composition is an indicator that can be used to discriminate between the types of fertilizer used: synthetic or organic. This element has two stable isotopes, ¹⁴N the most abundant and ¹⁵N [11]. Furthermore, nitrogen fertilization can impact the carbon cycle by directly influence the rate of photosynthesis and water efficiency [12]. Using organic fertilizer tends to increase the $\delta^{15}\text{N}$, reaching values around 10‰. In contrast, lower or negative values of $\delta^{15}\text{N}$ are found for synthetic fertilizers [13].

Considering that the type of fertilizer is determined by the cultivation system, meaning organic and biodynamic

use organic fertilization, while the conventional uses synthetic fertilizer. To differentiate the production system, the study aimed to investigate the impact of using organic and synthetic fertilizers on carbon and nitrogen isotopes in red and white grapes.

2. Materials and methods

2.1. Samples

The study was conducted with 120 samples, 60 organic (12 biodynamic) and 60 conventional, with red and white cultivars from different vineyards.

2.2. Experimental design

The experiment was designed to test the hypothesis that different agricultural management yield distinct isotopic signatures for nitrogen and carbon. This allows for the differentiation of organic, biodynamic, and conventional grape crops, thereby identifying the use of synthetic fertilizers during soil preparation for cultivation.

2.3. Measurements

The samples were dehydrated in an oven at a temperature of 70 °C for 24 hours for nitrogen determination. Then, 5 mg of the sample was placed in tin capsules and injected in the system using a solid sampler. The samples were introduced into the combustion reactor of the elemental analyser (Flash EA 1112, Thermo Fisher Scientific, Bremen, Germany), where they were converted into gas in a quartz reactor with copper oxide and cobalt and silver oxide, under a continuous flow of ultra-pure helium at a flow rate of 150 ml/min and pulses of oxygen for combustion.

The grape samples were manually crushed and placed in 2 mL vials for carbon determination. Afterward, 1 µL was injected using a liquid sampler following the method described at OIV-MA-AS312-06 [14].

2.4. Statistical analyses

Results were analysed with ByoEstat 5.3 at a 5% significance level, followed by Turkey's test.

3. Results and Discussion

Nitrogen can be derived from soil through plants and grapes, and exogenous sources strongly influence it during fertilization. These sources applied during the agriculture farming change the $\delta^{15}\text{N}$ as observed in the Figure 1. The average of $\delta^{15}\text{N}$ values for red and white organic grapes were $7.58 \pm 3.08\text{‰}$ and $8.93 \pm 0.14\text{‰}$, respectively. The conventional systems showed mean values of $2.32 \pm 1.09\text{‰}$ for red grapes and $3.08 \pm 1.06\text{‰}$ for white. Biodynamic grapes had an average of $6.93 \pm 3.09\text{‰}$.

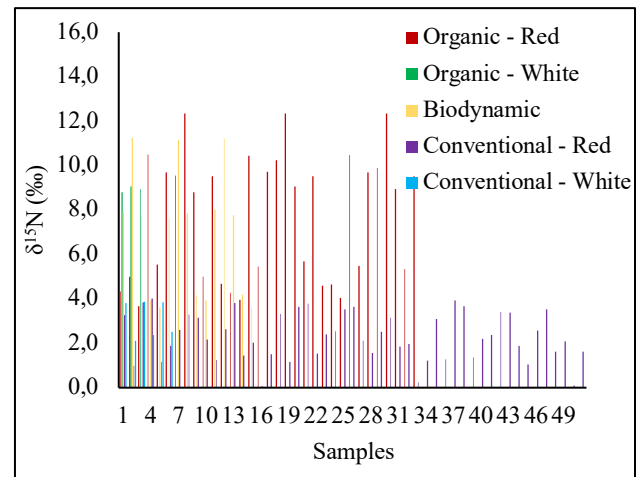


Figure 1. Results of $\delta^{15}\text{N}$ from organic, biodynamic and conventional management, for red and white grapes, expressed in ‰.

The $\delta^{15}\text{N}$ values for organic grapes in red and white cultivars were significantly higher than those conventional. While the average of $\delta^{15}\text{N}$ for white grapes was slightly higher than that for red grapes within organic and conventional groups, the differences were not statistically significant. Additionally, there were no significant differences between organic and biodynamic grapes systems.

The results are consistent with findings in the literature for both organic and conventional grapes [15, 16, 17]. Furthermore, they are aligned with the established threshold for vegetables in the literature, where $\delta^{15}\text{N}$ values for organic practices should exceed 5‰, while conventional practices generally have $\delta^{15}\text{N}$ values below 2‰ [18].

Synthetic fertilizers are widely used in conventional agriculture but are prohibited in organic farming. The nitrogen isotopic values of synthetic fertilizers ($\delta^{15}\text{N}_{\text{total}}$) are relatively uniform for all types, ranging from -1.7 to 3.9‰ with an average of 0.0‰ [19]. In contrast, organic fertilizers, derived from various sources, typically exhibited higher $\delta^{15}\text{N}$ values and a broader range of compositions than synthetic fertilizers. A plant's nitrogen isotope composition is influenced by the isotope signatures of external nitrogen sources and the plant's internal physiological process [20].

Achieving sustainability in grapevine cultivation demand careful management of water and fertilizers, especially nitrogen (N), to ensure the production of high-quality grapes for winemaking [21]. Over the years, studies have shown that grapevines use just 30 to 40% of the fertilizer, with the remainder becoming pollution [22].

Some $\delta^{15}\text{N}$ results in organic grapes showed intermediate values, closer to those of conventional grapes. These could be due to two factors. The first may relate to the transition process and the minimum time required to leach synthetic nitrogen from the soil, ensuring no further contamination from this fertilizer occurs. In such cases, continued monitoring is recommended. Suppose it is confirmed the transition period is insufficient to cleanse the soil. In that case, the official bodies should

be notified to review and potentially adjust the transition time to classify the practice as organic accurately. According to the official bodies, recommended period for converting a vineyard from conventional to organic is two to three years [23, 24].

The second possibility is that a mix of agricultural practices is occurring, meaning the producer is not fully adhering to all the principles of organic farming. The latter case could be considered a fraudulent practice, as it leads a consumer to believe the product is genuinely organic. Organic grapes are designated by a label that assures consumers the grapes were cultivated without addition of synthetic fertilizers and pesticides [5].

The stable isotope of carbon $^{13}\text{C}/^{12}\text{C}$ has also been investigated for organic authentication. In C_3 plants (Calvin Benson photosynthesis cycle), $\delta^{13}\text{C}$ is primarily influenced by water availability and drought stress. However, it is also significantly affected nutrient availability and fertilization practices, which often differ systemically between organic and conventional systems [25]. The results of $\delta^{13}\text{C}$ can be observed in Figure 2.

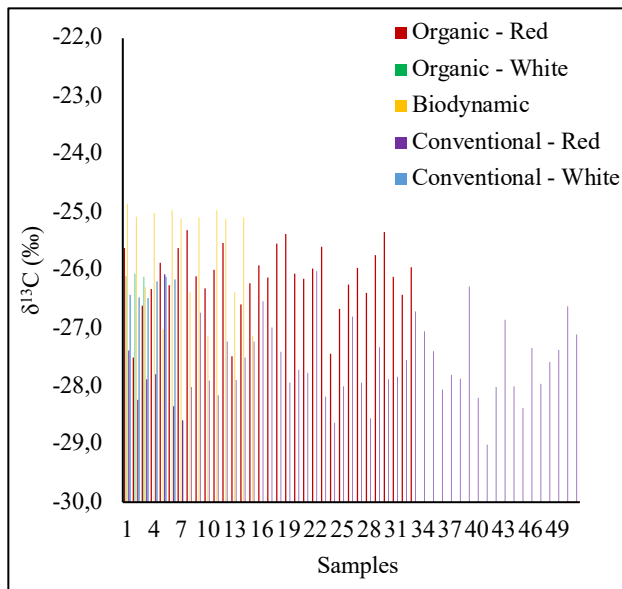


Figure 2. Results of $\delta^{13}\text{C}$ from organic, biodynamic and conventional management, for red and white grapes, expressed in ‰.

The average of $\delta^{13}\text{C}$ values for red and white organic grapes were $-26.14 \pm 0.61\text{‰}$ and $-26.09 \pm 0.03\text{‰}$, respectively. At the same time, the conventional systems showed mean values of $-27.60 \pm 0.67\text{‰}$ for red grapes and $-26.31 \pm 0.21\text{‰}$ for white. Biodynamic grapes had an average of $-25.71 \pm 0.97\text{‰}$.

Significant differences in $\delta^{13}\text{C}$ values were observed between organic and biodynamic for red cultivars compared to those from conventional systems. However, no significant differences were found between systems for white grapes. The $\delta^{13}\text{C}$ values are aligned with those reported in the literature [26, 27, 28]. The observed difference is likely due to the influence of nitrogen fertilization, which affects both the rate of photosynthesis and water use efficiency [29].

The $\delta^{13}\text{C}$ values were significantly lower than those from organic systems and are consistent with other studies [26, 30]. The photosynthetic pathway is the primary determinant of $\delta^{13}\text{C}$ signature. However, these results could be linked to the impact of biological respiration, specifically the decomposition of organic matter, on the isotopic composition of CO_2 within the microenvironment. Soil management techniques employed in organic systems, such as solarization and green manure, can increase soil respiration by enhancing microbial activity. This, in turn, may lower the $\delta^{13}\text{C}$ value of the soil CO_2 pool accessible to plants [26].

In conventional systems, $\delta^{13}\text{C}$ values were significantly higher for white cultivars than for red. This difference between red and white cultivars was not observed in organic systems. Similar differences in $\delta^{13}\text{C}$ values between varieties influenced by fertilization was already related in other studies [31].

4. Conclusion

The study successfully differentiated between organic, biodynamic, and conventional grape production systems based on nitrogen isotope composition, which varied significantly between the different fertilizer used. While carbon isotope is less effective for distinguishing between these systems, it is a good complement to reinforce the nitrogen result. This indicates that isotopes analysis, particularly nitrogen, is a promising approach for assessing and verifying the cultivation practices. Nitrogen isotope analysis proves to be a valuable tool for identifying production system.

5. Acknowledgments

This study was part of a research project supported by the Coordination for the Improvement of Higher Education Personnel (CAPES Foundation) with the Grant/Award Number: 88887.518542/2020-00, the University of Caxias do Sul (UCS), the Department of Agriculture of Rio Grande do Sul, the Cooperativa Vinícola Garibaldi, and SENS Advanced Mass Spectrometry.

6. References

1. O. Kaya, T. Yilmaz, F. Ates, F. Kustutan, H. Hatterman-Valenti, H. S. Hajizadeh, M. Turan, *Chem. Biol. Technol. Agric.* **11**, 38 (2024).
2. M. Krauss, A. Berner, D. Burger, A. Wiemken, U. Niggli, P. Mader, *Soil Use Mang.* **26**, 1 (2010).
3. D. R. Huggins, J. P. Reganold. *Sci. Am.* **96**, 6 (2008).
4. J. Döring, C. Collins, M. Frisch, R. Kauer. *Am. J. Enol.* **70**, 221 (2019).
5. A. Merot, N. Smits. *Eur. J. Agron.* **153**, 1 (2024).
6. OIV, I. O. (2021). Paris, France.

7. T. P. Cid, G. M. Santos, D. B. Azevedo. *Anais. Piracicaba*, SP (2023).
8. SISDEVIN. Sistema de declarações vinícolas (2023). <https://www.agricultura.rs.gov.br/sisdevin>
9. S. M. Krishnan, G. Barani. *Alochan Chakra J.* 1400 (2020).
10. G. Meissner, M. Athmann, J. Fritz, R. Kauer, M. Stoll, R. Shultz. *A.J.E.V.* **4**, 639 (2019).
11. J. Hoefs. *Stable Isotope Geochemistry*. Berlin, Heidelberg (2019).
12. V. H. Klaus, N. Hölzel, D. Prati, B. Schmitt, I. Schöning. *Plos One*, 8, 1 (2013).
13. F. A. Caxito, A. Silva. *Genomos*, **23**, 1 (2015).
14. OIV, I. O. (2024). Paris, France, 1732 p.
15. L. G. Santesteban, M. Loidi, I. Urretavizcaya, O. Oneka, D. Maín, A. Villa, J. B. Royo (2020).
16. C. T. Inácio, S. Urquiaga. *EMBRAPA*, p. 54 (2017).
17. V. L. Lima, C. Ducatti, P. R. Meirelles, L. C. Seraphin, M. A. Factori, A. M. Jorge, M. G. Silva. *Smin Cienc Agrar.* (2016).
18. J. M. Muñoz-Redondo, J. C. Montenegro, J. M. Moreno-Rojas. *Agronomy*, **13**, 1 (2023).
19. L. Vitória, N. Otero, N. Soler, A. Canals. **38**, 3254 (2004).
20. S. Verenitch, A. Mazumder. *Isot Enviro Healt S.*, **51**, 2, (2015).
21. F. V. Reluy, D. Intrigliolo, J. M. Miras-Avalos. *Aust J. Grape Wine Res.* 1, 7989254 (2023).
22. T. Verdenal, A. Dienes-Nagy, J. E. Spangenberg, V. Zufferey, J. L. Spring, O. Viret, C. Leeuwen. *IVES*, 1, 1 (2021).
23. USDA. Organic Regulations, 7 CFR Part 205 (2011).
24. Regulation (EU) 2018/848 of the European Union (2018).
25. M. Paolini, L. Ziller, D. Bertoldi, L. Bontempo, R. Larcher, G. Nicolini, F. Camin. *J. Mass Spectrom.* **51**, 9 (2016).
26. F. Longobardi, G. Casiello, V. Centonze, L. Catucci, A. Agostiano. *J. Sci. Food Agric.* **97**, 10 (2017).
27. L. Brillante, J. Martínez-Lüscher, R. Yu, S. K. Kurtural. *Front. Environ. Sci.* 8, 561477, (2020).
28. S. Leonardelli, J. G. Cargnel, F. R. Spinelli, R. Vanderlinde. *Rev. Bras. Vit. Eno.* 48, (2019).
29. V. H. Klaus, N. Hölzel, D. Prati, B. Schmitt, I. Schöning. *Plos One*. 8, 1 (2013).
30. M. Georgi, S. Vorkelius, A. Rossmann, J. Grassman, W. H. Schnitzler. *Plant Soil.* **275**, 93 (2005).
31. D. Taskos, E. Zioziou, N. Nikolaou, G. Doupis, S. Kounduras. *IVES*. 54, 4 (2020).