



Article

An Updated Isotopic Database of Fertilizers Used in Intensive Organic Farming: A Case Study on Protein Hydrolyzed Derivatives and Chelated Nutrients

José Manuel Muñoz-Redondo, Francisco Julián Cuevas, José Carlos Montenegro, José Luis Ordóñez-Díaz and José Manuel Moreno-Rojas *

Department of Agro-Industry and Food Quality, Andalusian Institute of Agricultural and Fisheries Research and Training (IFAPA), Alameda del Obispo, Avda Menéndez Pidal, s/n. 14004 Córdoba, Spain; josem.munoz.redondo@juntadeandalucia.es (J.M.M.-R.); fjulian.cuevas@juntadeandalucia.es (F.J.C.); josec.montenegro@juntadeandalucia.es (J.C.M.)

* Correspondence: josem.moreno.rojas@juntadeandalucia.es; Tel.: +34-671-53-27-58

Abstract: The global demand for organic food products has rapidly increased over the last years, becoming an emerging niche market targeting the high-income segment. The higher retailing price for organic food products may increase the risk of fraudulent practices at the different stages of the food supply chain, and consequently, substantial control is needed. Currently, the authentication of organic food products, such as those of plant origin, remains a key challenge in analytical chemistry. While stable isotopes have emerged as a powerful tool for this purpose, most studies have focused on crops, missing the agricultural inputs used for fertilization that influence the isotopic values of the crops. In this study, we aimed to isotopically characterize commonly used fertilizers, soil conditioners, and micronutrient fertilizers in intensive organic agriculture in the largest organic production region in the world (Almería, Spain). Our goal was to clarify the limitations that nitrogen isotopic fingerprinting presents for the fertilizer input industry and to characterize the organic inputs. The conventional fertilizers analyzed in this study showed low $\delta^{15}\text{N}$ values compared to their organic counterparts, except for some plant-based fertilizers, protein hydrolyzed fertilizers, and chelated nutrients. Both protein hydrolyzed fertilizers and micronutrient fertilizers presented a wide range of variability in their $\delta^{15}\text{N}$ values, including some very low or even negative values, more similar to those of conventional fertilizers. The results of this study highlight the challenges of authenticating organic foods in agriculture when using nitrogen isotope analysis.

Keywords: fertilizers; nitrogen stable isotopes; organic food authentication; soil fertility assessment; agricultural practices



Citation: Muñoz-Redondo, J.M.; Cuevas, F.J.; Montenegro, J.C.; Ordóñez-Díaz, J.L.; Moreno-Rojas, J.M. An Updated Isotopic Database of Fertilizers Used in Intensive Organic Farming: A Case Study on Protein Hydrolyzed Derivatives and Chelated Nutrients. *Horticulturae* **2024**, *10*, 523. <https://doi.org/10.3390/horticulturae10050523>

Academic Editors: Simona Fabroni, Francesco Montemurro and Luana Bontempo

Received: 9 April 2024

Revised: 6 May 2024

Accepted: 9 May 2024

Published: 18 May 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

The organic food industry has experienced substantial growth, with an estimated global market of EUR 106.4 billion in 2019 [1]. This expansion is largely driven by consumer demand for quality, sustainability, and food safety [2], making the consumers willing to pay more. However, the industry faces important challenges due to the lower agricultural yields of the organic farming systems compared to the conventional ones, implying higher retail prices. To address this challenge, the election of fertilizers in organic production has caught increasing attention as a solution to this yield gap. In this sense, the use of any agricultural input must be authorized by the appropriate inspection bodies, and regulatory frameworks are responsible for regulating the use of agricultural inputs in both organic and conventional farming. Examples include the European Council Regulation (EC) in Europe, the National Organic Standards Board (NOSB) in the United States, and the National Accreditation Services for Conformity Assessment in China. Currently, the European regulations address the harmonization of conditions for inorganic, organic, and

waste-based fertilizers within the internal European Union market. Nonetheless, some innovative organic fertilizers, such as protein hydrolyzed fertilizers (PHFs), have not been thoroughly addressed due to their rapid emergence in the market and their diverse origins. Consequently, individual national laws are required for each member state, making access to a single market more difficult due to the differing rules.

Protein hydrolyzed fertilizers have gained great attention among organic producers due to their valuable nutrients for plants, which are related with increases in soil fertility. Those characteristics have boosted a strong development of these agricultural inputs in organic management. PHFs are mainly produced by the denaturing and hydrolysis of various protein sources, such as plants, animals, or microorganism derivatives, leading to a wide diversity of origins and methods of synthesis. This varying composition makes it difficult to standardize their use by regulatory frameworks. Thus, PHFs were classified in the European laws as fertilizers and soil conditioners, as well as plant protection products. This latter classification makes their usage controversial, since no requirements for nitrogen concentration are needed. The same applies to the other class of organic fertilizers—micronutrients, which also do not require a nitrogen declaration. Therefore, more research is still required to clarify the origin of these fertilizers so that their legal status can be clearly defined and inspection bodies can ensure their appropriate use in organic crops.

Numerous analytical techniques have emerged over the recent years to address the differentiation between organically and conventionally grown crops [3], some of them based on the isotopic fingerprinting of the fertilizers used. For the authentication of organic foodstuffs, techniques based on isotope-ratio mass spectrometry have demonstrated their potential [4]. In this regard, the isotopic ratio of nitrogen of organic products has been related with the inputs used in agricultural systems [5], linking the soil and plant isotopic values of nitrogen [6] to fertilizers [7]. Particularly, several studies assessed the nitrogen isotopic values of fertilizers through correlations with nitrogen signatures found in fruits and vegetables in different agricultural systems [5]. This made it possible to discriminate between organic and conventional products, but it depended on different parameters that in many cases could not be controlled by the farmers, such as physiological processes. A previous study [8] stated that, although the nitrogen isotopic composition of the fertilizers used in conventional and organic systems were different, the variable impact can be reflected in the crop. This hypothesis was based on the broad range of environmental conditions and agricultural practices existing in the world and the fluctuating nitrogen cycle in different ecosystems. Therefore, it is necessary to gain an in-depth understanding of all the mechanisms influencing the isotopic signature of the products cultivated under different water and fertilizer regimes. Another important issue regarding these studies is the recent emergence of soil conditioners. To date, these agricultural inputs have not been isotopically characterized, but their nutrient content directly influences the absorption and bioavailability of the nitrogen supplied [9]. Soils have little capacity to retain oxidized forms of nitrogen and ammonium, and therefore, the accumulation of these types of fertilizers in soils is small. For this reason, most of the organic nitrogen fertilizers are associated with organic matter.

Considering the aforementioned, the aim of this study was to isotopically characterize the most commonly used fertilizers, soil conditioners, and micronutrient fertilizers in intensive organic agriculture in the largest organic production region in the world (Almería, Spain) to try to clarify the policy constraints that nitrogen isotopic fingerprinting presents for the fertilizer input industry. This will help create new databases to characterize organic inputs.

2. Materials and Methods

2.1. Samples

A total of 205 fertilizers (both liquid or solid) authorized for conventional ($n = 54$) and organic ($n = 151$) cultivation systems were obtained from different commercial fertilizer suppliers. We selected fertilizers that are commonly used in the region of Almería, aiming

to include a heterogeneous sample. This approach involved the selection of a variety of fertilizers with different compositions and suppliers. We consulted with local farmers and agricultural extension officers to identify the most widely used types of organic fertilizers. Detailed information about the origin and number of fertilizers by origin is shown in Table 1. These fertilizers are used in the systems under intensive conditions like the ones occurring in the south of Spain, including organic farming systems.

Table 1. Summary of the descriptive statistics for the fertilizers evaluated.

Synthetic fertilizers $\delta^{15}\text{N}$ (‰)							
Composition	Origin	Mean	SD	Max	Min	<i>n</i>	
Ammonia	Air	0.40	0.63	1.20	−0.30	8	
Ammonia + nitrate	Air	0.35	0.88	1.80	−0.50	16	
Ammonia + urea	Air	0.47	1.15	2.00	−1.20	12	
Ammonia + nitrate + urea	Air	0.47	0.29	0.80	0.30	6	
Nitrate	Air	−0.30	0.00	−0.30	−0.30	4	
Urea	Air	−0.23	0.68	0.30	−1.20	8	
Conventional organic fertilizers $\delta^{15}\text{N}$ (‰)							
Category	Type	Subtype	Mean	SD	Max	Min	<i>n</i>
Terrestrial	Animal	Blood	5.24	1.56	7.00	3.40	5
		By-products	7.97	4.32	12.31	1.88	7
		Horn	4.65	4.33	5.40	3.90	2
		Manure and slurry	11.21	3.14	14.40	5.15	8
	Plant	By-products	2.71	3.83	11.27	−1.63	23
Marine	Seaweed	3.67	2.62	6.10	0.90	3	
	Animal	2.30	0.57	2.70	1.90	2	
Organic fertilizers $\delta^{15}\text{N}$ (‰) (declaration of nitrogen content not required)							
PHF		3.41	3.10	10.35	−2.22	81	
Micronutrients		1.09	3.12	6.79	−2.34	20	

Both organic and inorganic fertilizers were processed similarly based on whether they were solid or liquid. All the liquid samples were homogenized by a vortex shaker for 1 min and sampled, and an aliquot of 2 mL was transferred to a vial and preserved in a freezer (−80 °C). All the liquid samples were weighed using 0.5 mg of Chemosorb© (nitrogen-free adsorber pad) and introduced into tin capsules. The Chemosorb© retained the fertilizer preventing losses of compounds due to the volatilization of the liquid samples, improving the repeatability of the results. Meanwhile, the solid samples were homogenized by grinding and milling in a ball mill for 60 s or more to obtain a fine powder. The organic fertilizers used in this approach were authorized by the European Union Council Regulation (Regulation (EC) No. 2018/848) and certification bodies.

2.2. Instruments and Standards

The choice of standards was based on the signal compatibility with the different types of fertilizers being analyzed (organic and synthetic). To achieve this, a series of preliminary tests were conducted to analyze the signal responses of various organic and inorganic fertilizers. Based on these tests, the standards used for synthetic fertilizers were IAEA-N1 (0.43‰), IAEA-N2 (20.30‰), IAEA-NO₃ (4.70‰), and Urea Isotopic Working Standard (−0.73‰) provided by the International Atomic Energy Agency (IAEA, Vienna, Austria) and IVA Analysentechnik e. K., (Dusseldorf, Germany). These standards are well established for covering a broad range of $\delta^{15}\text{N}$ values relevant to synthetic fertilizers [10]. To account for the signal responses observed in our tests, we added two additional standards for organic fertilizers: flour (sorghum flour, 1.58‰) and protein (casein, 5.94‰) standards provided by Elemental Microanalysis and IVA Analysentechnik e. K., respectively.

A Delta V Advantage Isotope Ratio Mass Spectrometer from Thermo Fisher Scientific (Bremen, Germany) equipped with a Flash EA elemental analyzer was used for the analysis of stable isotope ratios. The instrument was calibrated with the following international reference standards: IAEA-N1 (ammonium sulfate, $\delta^{15}\text{N} = 0.43\text{‰}$), IAEA-NO₃ (potassium nitrate, $\delta^{15}\text{N}$ value of -4.70‰), and IAEA-N2 (potassium nitrate, $\delta^{15}\text{N}$ value of 20.30‰). All the reference materials were certified by the International Atomic Energy Agency (IAEA, Austria) and used within the ranges 250–280 μg (except sorghum flour; 700–1000 μg) to achieve a signal intensity between 2700 and 6000 mV. The long-term standard deviation of their values was lower than 0.15‰ .

2.3. Determination of $^{15}\text{N}/^{14}\text{N}$ Isotope Ratio by EA-IRMS

The samples were weighed into tin capsules ($3.3 \times 5\text{ mm}$, IVA Analysentechnik e. K., Dusseldorf, Germany).

The carrier and reference gases (He) were circulated at 140 and 200 mL min^{-1} , respectively. The temperatures of the elemental analyzer were set at 65 and 1020 $^{\circ}\text{C}$ and 650 $^{\circ}\text{C}$ for the oven and the reduction and oxidation reactors, respectively. The results of the nitrogen isotope ratio ($\delta^{15}\text{N}$) analyses were reported as the per mille (‰) enrichment relative to the international standard (air nitrogen) for the nitrogen isotope ratio according to Equation (1):

$$\delta^{15}\text{N}_{\text{sample}}(\text{‰}) = [(R_{\text{sample}} - R_{\text{standard}})/R_{\text{standard}}] \times 1000 \quad (1)$$

where R_{sample} and R_{standard} denote the ratio between the heavier and the lighter isotope ($^{15}\text{N}/^{14}\text{N}$) in the sample and reference material, respectively.

The fertilizers were analyzed in duplicate with variability below 0.3‰ for $\delta^{15}\text{N}$ measurements as previously reported [7].

In the same analysis, the percentage of nitrogen was measured by the elemental analyzer. All the commercial fertilizers presenting a declared nitrogen content were checked, with no significant deviation compared to the nitrogen declared. The box and whisker plot was constructed using SPSS© software (v. 17.0, IBM, Chicago, IL, USA).

2.4. Statistical Analyses

An analysis of variance (ANOVA) was conducted to determine the differences among the various classes of fertilizers. To further compare the means of the different groups, the Tukey's honestly significant difference (Tukey HSD) test was used as a post hoc analysis. This allowed us to determine which groups exhibited statistically significant differences in their means. The differences were considered statistically significant at a p -value < 0.05 .

3. Results and Discussion

3.1. Synthetic Fertilizers

Synthetic fertilizers are produced from the fixation of atmospheric nitrogen into ammonium through a chemical reaction known as the Haber–Bosch process [11]. This process does not cause significant N isotope fractionation, although slight fractionation may occur in subsequent processes such as the transformation of NH_4^+ into NO_3 [12]. Therefore, synthetic fertilizers are expected to display $\delta^{15}\text{N}$ values close to that of the atmospheric nitrogen (0‰). In this sense, these fertilizers displayed homogeneous $\delta^{15}\text{N}$ values (Figure 1) that ranged between -1.2 and 2.0‰ , with a mean value of 0.26‰ and a standard deviation of 0.8‰ . These results were in accordance with previous studies that showed a similar range of variation [5,7]. When assessing the results of inorganic and organic fertilizers, a lower range of variation was observed in the inorganic fertilizers (Figure 1). An analysis of variance (ANOVA) was performed to compare the two types of fertilizers, obtaining statistically significant differences at a p -value < 0.001 . The mean value for organic fertilizers was 3.69‰ , in comparison with 0.26‰ obtained for inorganic

fertilizers. These results were as expected, since it has already been demonstrated that inorganic fertilizers show lower $\delta^{15}\text{N}$ values than their organic counterparts [7].

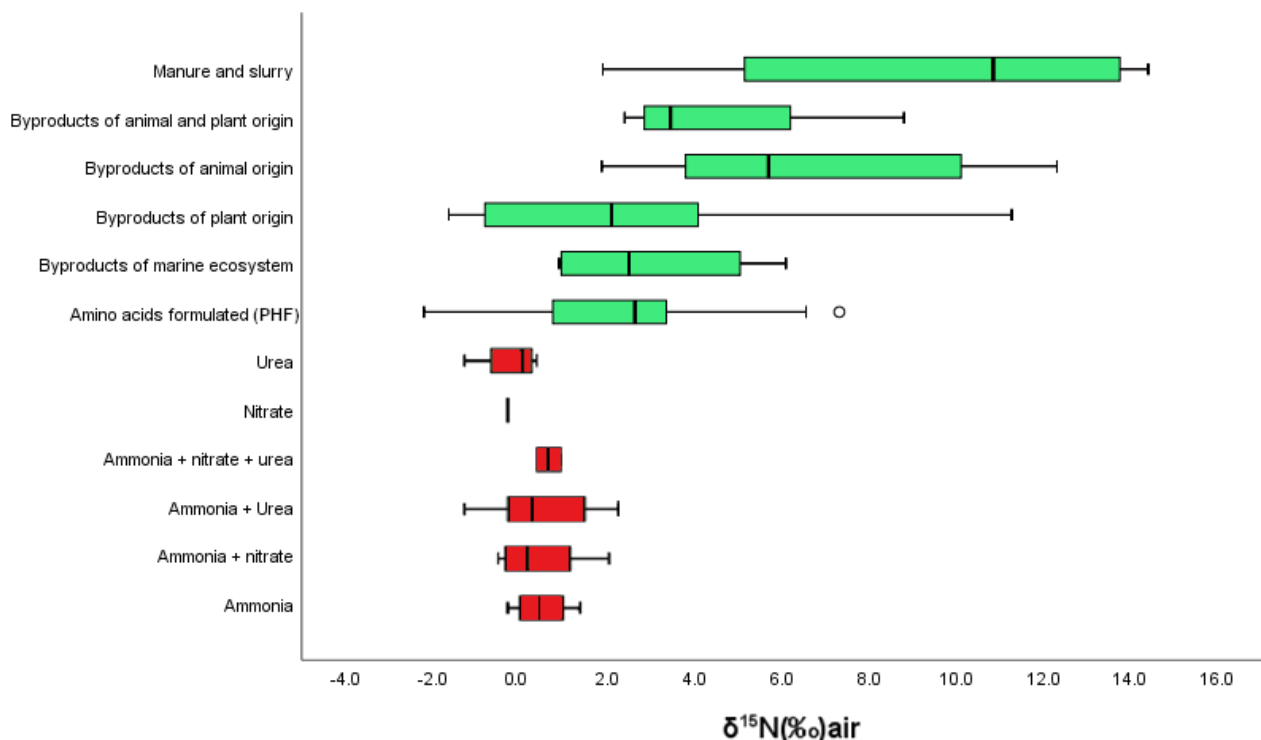


Figure 1. Box and whisker plot for the distribution of $\delta^{15}\text{N}$ values of conventional and organic fertilizers used in intensive conventional (red) and intensive organic (green) agricultural systems.

3.2. Organic Fertilizers

The wide diversity of organic fertilizers makes their classification complex, although two main categories can be distinguished according to their ecosystem origin: marine and terrestrial. These categories were further categorized in different subtypes, as shown in Table 1. The $\delta^{15}\text{N}$ (‰) value for terrestrial ecosystems ranged between a minimum of -1.63 ‰ and a maximum of 14.40 ‰. The lower values belonged to plant by-products and the maximum ones to animal-based fertilizers. The plant by-products showed high variability in relation to the different ways in which nitrogen is incorporated into the plants. The plant $^{15}\text{N}/^{14}\text{N}$ ratio is an integrator of the nitrogen cycle between soil and air, which can be affected by several processes, such as plant fertilizer sources, symbiotic atmospheric fixation, mycorrhizal symbioses, or fractionation during uptake [13]. Within the terrestrial ecosystem, the animal samples were more enriched in ^{15}N compared to the plants. The highest $\delta^{15}\text{N}$ values were detected in manure and slurry samples (Figure 1) in agreement with the previous literature [6,7]. Moreover, the $^{15}\text{N}/^{14}\text{N}$ ratio of an animal-based fertilizer is highly dependent on the trophic level of the animal from which it derives. Additionally, Choi et al. [14] reported that the isotopic composition of fresh animal excrement is not usually different from the $\delta^{15}\text{N}$ of synthetic fertilizers. Thus, the main fractionation vector in manure is maturity, due to its exposure to the atmosphere and the speedy loss of $^{14}\text{NH}_3$ via volatilization [15]. These authors reported that the $\delta^{15}\text{N}$ of total N of cattle manure compost increased rapidly within 10 days of composting, but further increases were not observed thereafter in the following 90 days of composting, suggesting that the ^{15}N enrichment of composted manure is a fast process occurring in the early stage of composting and that it is affected by the bedding (sawdust, crop residues, etc.) material [15]. The mean values for the blood samples reported in our study were about 5.24 ‰ (Table 1). The animal by-products category was mainly characterized by meat powder samples with a high variability, values

of $\delta^{15}\text{N}$ ranging from 1.88‰ to 12.31‰. Moreover, the mean value for fertilizers based on horns was slightly slower (4.65‰) than that reported by other authors [7] (around 6‰).

Our results for marine fertilizers were similar to the $\delta^{15}\text{N}$ values previously obtained in the literature [7]. The values of this type of organic inputs varied from 0.9‰ to 6.10‰, displaying slightly higher values than inorganic fertilizers (Figure 1). Seaweed cannot fix nitrogen and first take up nitrogen as nitrate and ammonium. These seaweeds extracts are growing in popularity among farmers in intensive agriculture due to their enhanced abiotic stress tolerance, growth, nutritional quality, and postharvest quality, which have been recently reviewed [16]. One of the main advantages of these fertilizers is the fact that they are available as a liquid extract or in soluble powder forms. This fact makes it possible to mix the extracts with irrigation water that can be easily applied by drip irrigation in intensive greenhouses.

3.2.1. Protein Hydrolyzed Fertilizers (PHFs)

Protein hydrolyzed fertilizers are allowed to be used in organic farming, as shown in Figure 2A. The European regulation allows their use either as fertilizers, soil conditioners, or nutrients, where the % N needs to be declared, or as plant protection products, where the % N does not need to be declared. This lack of requirement for declaring the nitrogen content in plant protection products may make their use controversial, highlighting the need for in-depth research to provide greater clarity in regulatory standards.

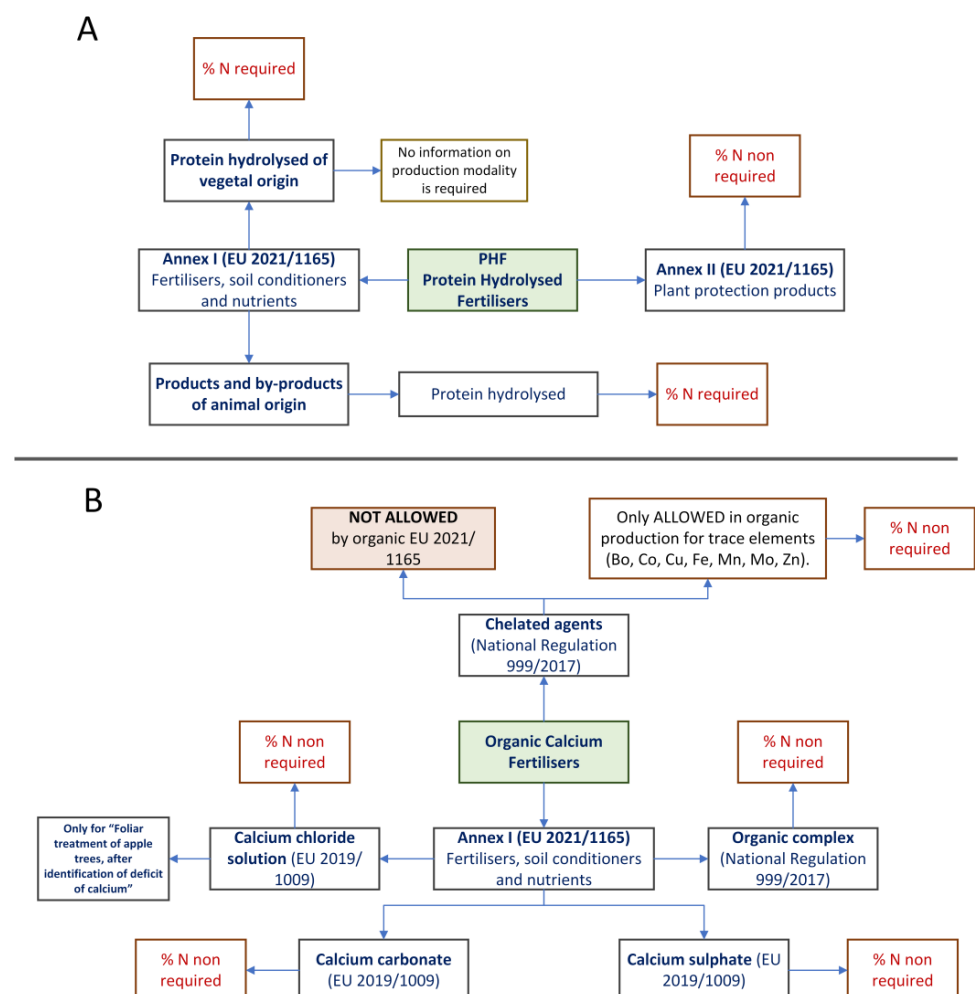


Figure 2. Authorized formulations of protein hydrolyzed fertilizers (A) and micronutrients in organic farming (B).

The PHFs analyzed in this study showed a high variability in their $\delta^{15}\text{N}$ values due to their different origins and the production techniques used, with a mean $\delta^{15}\text{N}$ value of 3.41‰ and a distance between the maximum (10.35‰) and minimum (−2.22‰) values of 12.57‰ (Table 1). This was explained by the low (even negative) $\delta^{15}\text{N}$ values of several PHFs, which were in the same range as the synthetic fertilizers (Tables 1 and 2). Low $\delta^{15}\text{N}$ values were predominantly found in PHFs derived from leguminous plants. This could be attributed to the capacity of leguminous plants to fix atmospheric nitrogen (which has $\delta^{15}\text{N}$ values close to 0), minimizing the isotopic fractionation [13] and resulting in lower $\delta^{15}\text{N}$ values in their derived fertilizers. From the analysis of variance (ANOVA), we observed a clear differentiation of PHFs according to their origin at a p -value < 0.001 (Table 2). The hydrolyzed fertilizers from vegetal material clearly displayed lower $\delta^{15}\text{N}$ values (with a mean of 2.25‰) than the animal-origin counterparts (6.27‰). Figure 3 illustrates the distribution of these values, revealing a separation between animal- and plant-derived PHFs at $\delta^{15}\text{N}$ values of approximately 3.8‰. Consequently, depending on the management practices employed, the application of plant-based PHFs with low isotopic values could influence organic crop cultivars by modifying the $\delta^{15}\text{N}$ values in cultivated plants and fruits. This reduction could result in isotopic signatures typically observed in conventional crops. These results, combined with the fact that regulations do not require the declaration of the nitrogen content in these types of fertilizers when used as plant protection products, could make their use controversial under the current regulatory framework when using $\delta^{15}\text{N}$ to certify the organic origin of products. Consequently, this study highlights the need for research on the impact of PHFs with low $\delta^{15}\text{N}$ values on the isotopic values of the crops.

Table 2. Mean and deviation for the $\delta^{15}\text{N}$ and % N of PHFs according to their hydrolysis type and origin. Results for the analysis of variance (ANOVA) are also shown.

Factor	PHF	$\delta^{15}\text{N}$ Mean (‰)	$\delta^{15}\text{N}$ SD (‰)	p -Value (ANOVA)	% N Mean (‰)	% N SD (‰)	p -Value (ANOVA)
Origin	Animal (N = 15)	6.27	3.08	<0.001	7.33	4.18	<0.05
	Vegetal (N = 30)	2.25	2.63		4.59	4.24	
	Unknown (N = 36)	3.18	2.78	-	6.65	4.73	-
Hydrolysis	Acid (N = 10)	3.83	3.35	ns	8.13	7.00	ns
	Enzymatic (N = 51)	2.89	2.42		5.58	4.23	
	Unknown (N = 20)	4.51	4.21	-	6.06	3.71	-

ns = not significant

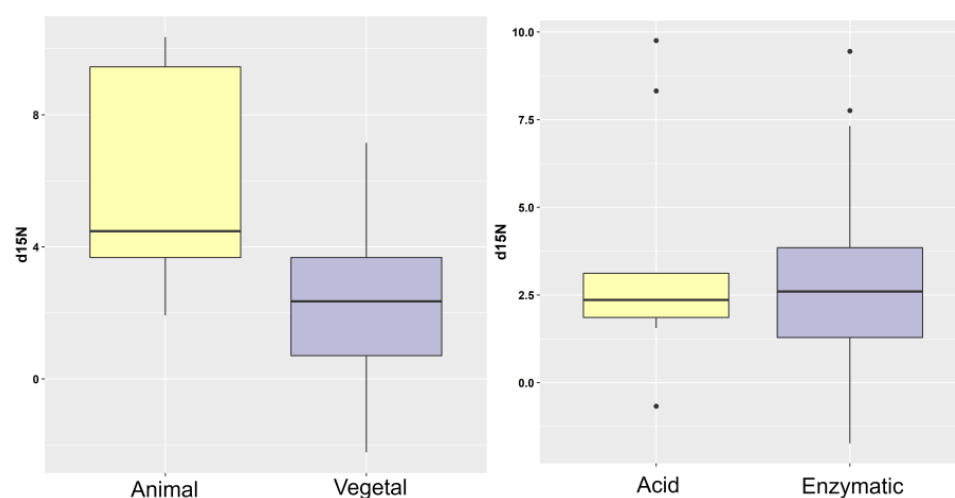


Figure 3. Box and whisker plot for the distribution of $\delta^{15}\text{N}$ values of PHFs from different origins (plant or animal; left) and hydrolysis methods (acid or enzymatic; right).

Significant differences were also found in the % N between PHFs from vegetal and animal origin, with the latter showing a higher percentage of nitrogen (Table 2) and highlighting the influence of the source material on the final PHF composition. Additionally, different hydrolysis processes, either acid or enzymatic, can be used for production of organic fertilizers, which may potentially cause different fractionation patterns. However, no statistically significant differences were found in the $\delta^{15}\text{N}$ values between the fertilizers obtained under these production techniques, as shown in Table 2 and Figure 3.

3.2.2. Micronutrients

Another type of fertilizer permitted in organic farming is micronutrients, which include calcium and trace element formulations as shown in Figure 2B. These do not require a declaration of the nitrogen content either, although some of them have shown high total nitrogen levels and low $\delta^{15}\text{N}$ values (Table 1). Chelated micronutrients are fertilizers with essential trace elements chemically bonded with a chelating agent. This enhances the bioavailability and solubility of the micronutrients, making them more accessible to plants. Examples of chelated micronutrients include chelated forms of iron, zinc, manganese, and copper, among others. The chelating agent helps prevent the micronutrients from binding with other substances in the soil, allowing the plants to absorb them more effectively. These properties make them widely used in agriculture. Moreover, the addition of certain carbon and nitrogen substrates can influence soil organic matter decomposition and N_2O emissions [17], which may have implications for the effectiveness and environmental impact of using these types of fertilizers in organic farming.

Among the different nutrients, calcium is essential for crops, especially in greenhouses in Almería, where ‘blossom-end rot’ due to low calcium levels in nutrient solutions [18] is a common issue for tomatoes and peppers. Farmers and previous research also recognized the importance of other trace elements (B, Zn, Mo, etc.) in organic farming nutrition to avoid deficiencies and promote plant growth [19].

Twenty commercial micronutrient fertilizers were characterized, of which five had a total nitrogen content below 0.5%, eleven ranged between 1 and 5%, and four displayed values above 5% (Table 3). The highest nitrogen content was observed in formulations with chelated calcium and trace elements. The $\delta^{15}\text{N}$ values of the micronutrients analyzed ranged from -2.34 to 9.48‰ , averaging 2.4‰ , which is more similar to values observed in conventional fertilizers. In fact, half of the fertilizers analyzed had $\delta^{15}\text{N}$ values below 0‰ . Consequently, no statistically significant differences ($p\text{-value} > 0.05$) were found from an analysis of variance (ANOVA) comparing these fertilizers with the conventional ones. Similar to the PHFs, certain fertilizers allowed in organic farming exhibited $\delta^{15}\text{N}$ values and high percentages of total nitrogen, which did not need to be declared according to regulations. This raises the need for further research to determine whether these fertilizers could impact the authentication of organic products when $\delta^{15}\text{N}$ is used for verification.

Table 3. Categories, declared elements, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values, and percentage of nitrogen in chelated nutrients.

Category	Declared Elements	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	%N
Chelated calcium	Ca, EDTA	-0.57	-26.91	9.78
Trace element	Fe, Mn, Zn, B, Cu, EDTA	-0.57	-27.20	8.28
Chelated calcium	Ca	0.25	-30.01	7.42
Trace element	Zn, EDTA	-1.14	-31.01	6.88
Trace element	Bo	-2.34	-30.25	4.99
Organic complex	Ca	1.11	-30.41	4.89
Trace element	Zn, Fe, B, Mo	4.15	-30.01	4.82
Trace element	Zn, B, Mo	-1.87	-26.78	3.43
Calcium chloride	Ca, Mat.Org	3.36	-19.33	2.48
Trace element	B, Ma, Mn	9.02	-26.06	1.97
Calcium carbonate	Ca, Mg	3.22	-22.84	1.91

Table 3. Cont.

Category	Declared Elements	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	%N
Calcium carbonate	Ca, Mg	2.76	-	1.78
Trace element	Zn, B, Mo	-0.62	-27.41	1.45
Trace element + chelated calcium	Ca, Mg, Fe	9.48	-24.62	1.29
Trace element	Zn, Fe, B Mo	-0.88	-24.63	1.12
Calcium chloride	Ca	7.73	-7.31	0.43
Calcium sulfate	Ca, Mg	-0.21	-	0.10
Calcium chloride	Ca	6.79	-3.34	0.09
Calcium chloride	Ca	-0.88	-17.65	0.04
Trace element + calcium chloride	Zn, Ca	-0.19	-	0.03

4. Conclusions

This study reflects the importance of deepening research into the limitations of stable isotopes for the traceability of organic and conventional foodstuffs. The conventional fertilizers analyzed in this study showed low $\delta^{15}\text{N}$ values compared to their organic counterparts, except for some plant-based fertilizers, protein hydrolyzed fertilizers, and chelated nutrients. Both protein hydrolyzed fertilizers and micronutrient fertilizers presented a wide range of variability in their $\delta^{15}\text{N}$ values, including some low or even negative values. Additionally, several of both types of fertilizers showed a high nitrogen content, which does not need to be declared according to regulations (in the case of chelating agents and protein hydrolyzed fertilizers when used as plant protection products). Consequently, the use of these fertilizers may present challenges in authenticating organic products and the likely impact of their $\delta^{15}\text{N}$ values. Therefore, based on the results of this study, it is essential to conduct further research on how factors affecting the fertilizers' isotopic composition impact crops and the final food products.

Author Contributions: Conceptualization, J.M.M.-R. (José Manuel Moreno-Rojas); methodology, J.M.M.-R. (José Manuel Moreno-Rojas), J.M.M.-R. (José Manuel Muñoz-Redondo), F.J.C. and J.C.M.; software, J.M.M.-R. (José Manuel Muñoz-Redondo) and F.J.C.; formal analysis, J.M.M.-R. (José Manuel Muñoz-Redondo); investigation, J.M.M.-R. (José Manuel Moreno-Rojas), J.M.M.-R. (José Manuel Muñoz-Redondo), F.J.C., J.L.O.-D. and J.C.M.; resources, J.M.M.-R. (José Manuel Moreno-Rojas); data curation, J.M.M.-R. (José Manuel Moreno-Rojas), J.M.M.-R. (José Manuel Muñoz-Redondo), F.J.C. and J.C.M.; writing—original draft preparation, J.M.M.-R. (José Manuel Muñoz-Redondo), J.M.M.-R. (José Manuel Moreno-Rojas), and F.J.C.; writing—review and editing, J.M.M.-R. (José Manuel Moreno-Rojas), J.L.O.-D. and J.M.M.-R. (José Manuel Muñoz-Redondo); supervision, J.M.M.-R. (José Manuel Moreno-Rojas); project administration, J.M.M.-R. (José Manuel Moreno-Rojas); funding acquisition, J.M.M.-R. (José Manuel Moreno-Rojas). All authors have read and agreed to the published version of the manuscript.

Funding: This work was funded by the Andalusian Institute of Agricultural and Fisheries Research and Training (IFAPA, Spain) through the projects 'Evaluación de la trazabilidad de nuevos insumos derivados de hidrolizados de proteínas (DHP) utilizados en agricultura ecológica respecto al origen de los productos de partida, metodologías de síntesis y su integridad (FERTI-TRACE)' (PP.AVA.AVA2019.045) and 'Viabilidad de la relación de isótopos estables de nitrógeno ($^{15}\text{N}/^{14}\text{N}$) como metodología para la caracterización de la producción ecológica frente a la convencional' (PR.PEL.IDF2016.01), and the European Rural Development Fund (ERDF, EU). J.M.M.-R. (José Manuel Muñoz-Redondo) and J.C.M. received grants through a research contract at IFAPA funded through the projects PP.AVA.AVA2019.045 and PR.PEL.IDF2016.01, respectively.

Data Availability Statement: Data are contained within the article.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Willer, H.; Trávníček, J.; Meier, C.; Schlatter, B. *The World of Organic Agriculture. Statistics and Emerging Trends*; IFOAM: Bonn, Germany, 2021; p. 340.
2. Baron, J.; Jay-Russell, M.T. Assessment of Current Practices of Organic Farmers Regarding Biological Soil Amendments of Animal Origin in a Multi-Regional US Study. *Food Prot. Trends* **2018**, *38*, 347–362.
3. Capuano, E.; Boerrigter-Eenling, R.; van der Veer, G.; van Ruth, S.M. Analytical Authentication of Organic Products: An Overview of Markers. *J. Sci. Food Agric.* **2013**, *93*, 12–28. [[CrossRef](#)] [[PubMed](#)]
4. Laursen, K.H.; Schjoerring, J.K.; Kelly, S.D.; Husted, S. Authentication of Organically Grown Plants—Advantages and Limitations of Atomic Spectroscopy for Multi-Element and Stable Isotope Analysis. *TrAC Trends Anal. Chem.* **2014**, *59*, 73–82. [[CrossRef](#)]
5. Inácio, C.T.; Chalk, P.M.; Magalhães, A.M. Principles and Limitations of Stable Isotopes in Differentiating Organic and Conventional Foodstuffs: 1. Plant Products. *Crit. Rev. Food Sci. Nutr.* **2015**, *55*, 1206–1218. [[CrossRef](#)] [[PubMed](#)]
6. Chalk, P.M.; Magalhães, A.M.; Inácio, C.T. Towards an Understanding of the Dynamics of Compost N in the Soil-Plant-Atmosphere System Using ^{15}N Tracer. *Plant Soil* **2013**, *362*, 373–388. [[CrossRef](#)]
7. Bateman, A.S.; Kelly, S.D. Fertilizer Nitrogen Isotope Signatures. *Isotopes Environ. Health Stud.* **2007**, *43*, 237–247. [[CrossRef](#)] [[PubMed](#)]
8. Bateman, A.S.; Kelly, S.D.; Woolfe, M. Nitrogen Isotope Composition of Organically and Conventionally Grown Crops. *J. Agric. Food Chem.* **2007**, *55*, 2664–2670. [[CrossRef](#)] [[PubMed](#)]
9. Fageria, N.K.; Baligar, V.C. Enhancing Nitrogen Use Efficiency in Crop Plants. *Adv. Agron.* **2005**, *88*, 97–185.
10. Gentile, N.; Rossi, M.J.; Delémont, O.; Siegwolf, R.T.W. $\delta^{15}\text{N}$ Measurement of Organic and Inorganic Substances by EA-IRMS: A Speciation-Dependent Procedure. *Anal. Bioanal. Chem.* **2013**, *405*, 159–176. [[CrossRef](#)] [[PubMed](#)]
11. Schlesinger, W.H. On the Fate of Anthropogenic Nitrogen. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 203–208. [[CrossRef](#)] [[PubMed](#)]
12. Freyer, H.D.; Aly, A.I.M. Nitrogen-15 Variations in Fertilizer Nitrogen†. *J. Environ. Qual.* **1947**, *3*, 405–406. [[CrossRef](#)]
13. Robinson, D. $\delta^{15}\text{N}$ as an Integrator of the Nitrogen Cycle. *Trends Ecol. Evol.* **2001**, *16*, 153–162. [[CrossRef](#)] [[PubMed](#)]
14. Choi, W.-J.; Kwak, J.-H.; Lim, S.-S.; Park, H.-J.; Chang, S.X.; Lee, S.-M.; Arshad, M.A.; Yun, S.-I.; Kim, H.-Y. Synthetic Fertilizer and Livestock Manure Differently Affect $\delta^{15}\text{N}$ in the Agricultural Landscape: A Review. *Agric. Ecosyst. Environ.* **2017**, *237*, 1–15. [[CrossRef](#)]
15. Kim, Y.-J.; Choi, W.-J.; Lim, S.-S.; Kwak, J.-H.; Chang, S.X.; Kim, H.-Y.; Yoon, K.-S.; Ro, H.-M. Changes in Nitrogen Isotopic Compositions during Composting of Cattle Feedlot Manure: Effects of Bedding Material Type. *Bioresour. Technol.* **2008**, *99*, 5452–5458. [[CrossRef](#)] [[PubMed](#)]
16. Battacharyya, D.; Babgohari, M.Z.; Rathor, P.; Prithiviraj, B. Seaweed Extracts as Biostimulants in Horticulture. *Sci. Hortic.* **2015**, *196*, 39–48. [[CrossRef](#)]
17. Li, Y.; Moinet, G.Y.; Clough, T.J.; Whitehead, D. Organic Matter Contributions to Nitrous Oxide Emissions Following Nitrate Addition Are Not Proportional to Substrate-Induced Soil Carbon Priming. *Sci. Total Environ.* **2022**, *851*, 158274. [[CrossRef](#)] [[PubMed](#)]
18. Saure, M.C. Why Calcium Deficiency Is Not the Cause of Blossom-End Rot in Tomato and Pepper Fruit—A Reappraisal. *Sci. Hortic.* **2014**, *174*, 151–154. [[CrossRef](#)]
19. Wyszowski, M.; Brodowska, M.S. Content of Trace Elements in Soil Fertilized with Potassium and Nitrogen. *Agriculture* **2020**, *10*, 398. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.