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How Much Do Soil and Water Contribute to the Composition of Meat? A Case Study: Meat from Three Areas of Argentina

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Supporting Information

ABSTRACT: The main goal of this study was to propose a reliable method to verify the geographical origin of meat, establishing the influence of soil and water on its isotopic and elemental composition. Thus, beef meat, soil, and water samples were collected from three major cattle-producing regions of Argentina (Buenos Aires, Córdoba, and Entre Ríos). Multielemental composition was determined on these three matrices by inductively coupled plasma mass spectrometry (ICP-MS), $\delta^{13}C$ and $\delta^{15}N$ by isotope-ratio mass spectrometry (IRMS), and the $^{87}Sr/^{86}Sr$ ratio by thermal ionization mass spectrometry (TIMS). Soil and drinking water samples could be characterized and clearly differentiated by combining the isotopic ratios and elements, demonstrating differences in geology and climatic conditions of three regions. Similarly, meat originating at each sampling area was characterized and differentiated using only five key variables (Rb, Ca/Sr, $\delta^{13}C$, $\delta^{15}N$, and $^{87}Sr/^{86}Sr$). Generalized procrustes analysis (GPA), using the three studied matrices (soil, water, and meat) shows consensus between them and clear differences between studied areas. Furthermore, canonical correlation analysis (CCA) demonstrates significant correlation between the chemical-isotopic profile of meat with those corresponding to both soil and water ($r^2 = 0.93$, $p < 0.001$; and $r^2 = 0.83$, $p < 0.001$, respectively). So far, there are clear coincidences between the meat fingerprint and those from soil/water where cattle grew, presenting a good method to establish beef provenance. To the authors' knowledge this is the first report linking the influence of soil and water all together on the composition of beef, presenting the basis for the authentication of Argentinean beef, which could be extended to meat from different provenances.

KEYWORDS: beef, trace elements, isotopic composition, geographical origin, authenticity

INTRODUCTION

In recent years, research efforts have focused on the potential of analytical techniques to assess the geographical origin of agricultural products.[1,2] The need to absolutely identify the provenance of foods has been increased as a consequence of the expansion in global trade, with the aims to increase confidence in the provenance of foods from the desired region and avoid false declarations of origin, frauds, use of third countries to allow the exportation from banned areas, etc. In particular, the trade of cattle products, such as beef and dairy products, has received more attention than previously mainly due to concerns relating to bovine spongiform encephalopathy (BSE).[3] The trade of beef is intensively controlled by a registration system of animals. However, the BSE crisis and periodic episodes of foot and mouth disease (FMD) have deteriorated the confidence of regulatory authorities and consumers as well. Furthermore, if the animal is slaughtered, resulting in meat pieces, the evaluation of its origin by registration methods diminishes.

To control the authenticity and the provenance of meat, the use of stable properties is mandatory, in addition to widely accepted standard methods of analysis and, in many cases, international and local regulations.[4] Measurements of trace elements and stable isotopic ratios by inductively coupled plasma mass spectrometry (ICP-MS) are predominantly used for the authentication of geographic origin.[5,6] However, many scientific reports are focused on differences in the product itself, pointing out diverse elements/parameters that allow the classiffication of foods from several origins. Studies linking the characteristics of soil, water, and environment from the original area with those of the food product analyzed are few.[3,7,8]

The assessment of the geographic origin of meat, based on analytical tools, seems to be possible considering that cattle

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feeding is influenced by geographic and environmental factors. However, the demonstration of specific links between the geography and its associated geology, considering environmental issues and feeding practices, is still a challenge. There are at least two types of indicators to assess the geographic origin of meat. Primary indicators are directly related to the area where animals grew, considering that changes can occur from birth to submission to slaughterhouses. Examples of primary indicators are isotopic ratios, that is, δD and δ^{18}O from drinking water and δ^{15}N, δ^{34}S, the ^{87}Sr/^{86}Sr ratio, and trace elements from the soil and the environment. Secondary indicators are linked to production systems, including animal genotype, feeding type and conditions, and exposure to different microbials, which are supposed to be associated with certain regions but which may change for various reasons. In some cases the determination of the origin through primary indicators might be sufficient, whereas in other cases secondary indicators must also be considered to get a reliable fingerprint from which the provenance of meat can be assessed.[9]

The analysis of trace elements is considered to be an effective primary tool, mainly because the elemental composition from the local environment (soil, drinking water, etc.) can be reflected in agricultural products, whereas differences in the distribution of these trace elements among different geographical locations can give various element signatures in organic tissues.[10] The multielemental composition of animal tissues reflects, to some extent, that of the vegetation and water consumed.[1,9] The vegetation is the compositional reflection of the bioavailable and mobilized nutrients present in the underlying soils. The availability of trace elements depends on several factors such as soil, pH, humidity, porosity, clay and humic complex, etc.[11] Although the analysis of trace elements has been widely used for food authentication,[12−15] the use of multielement analysis as origin tracers for animal products is limited because the elemental composition in animal tissues is influenced by various factors. Inconclusive results may occur when animals switch between areas during fattening or in response to the use of supplements in animal feed. Furthermore, it must be taken into account that animal tissues have different capabilities to accumulate elements.[9,10]

The isotopic composition could be considered as a natural property that can be changed by incomplete turnover processes only; therefore, it may serve as a kind of "natural fingerprint". Organisms more or less reflect the isotopic composition of different materials in their environment, for example, sulfur, carbon, or nitrogen.[4] Variation from natural stable isotopic composition is currently used as a valuable tool to control the authenticity of many products (cane sugar added to wine or honey, organic vs nonorganic cultivars, etc.). This technique is based on the small but significantly different ratios of stable isotopes of bioelements, mainly hydrogen, oxygen, carbon, and nitrogen, present in certain organic molecules. Such differences arise from kinetic-chemical and physical factors that can be correlated with the botanical-metabolic and/or geographical origin of a product, respectively, for instance, carbon discrimination against ^{13}C in C3 plants with respect to C4 plants[16] or radiogenic decay from Rb to ^{87}Sr,[17] etc. The ratios ^{1}H/^{2}H and ^{16}O/^{18}O in body tissues are primarily influenced by drinking water. Isotopic ratios of C, N, S, and Sr are more indicative of soil and feed origin.[1,5,9] In general, stable carbon and nitrogen isotopic composition of animal tissue reflect those of the diet. The carbon isotopic composition of meat and dairy products reflects the proportion of C3 plants (e.g., clover) and C4 plants (e.g., maize) in the diet.[1,3−5] The ^{15}N/^{14}N ratio in the biomolecules depends on the ^{15}N content of the inorganic nitrogen present in the soil (the primary source of nitrogen for plants). This in turn depends on the type of fertilizer used, because, for instance, organic fertilizers and intensive farming methods increase the level of ^{15}N in the soil.[18] An especially promising isotopic ratio to determine the geographic origin of meat could be that of Sr, as it is typical for soil of certain regions. The natural isotopic composition of strontium varies with ^{87}Rb decay and, as a consequence, the local ratio of ^{87}Sr/^{86}Sr depends on geological setting. As Kelly et al.[1] pointed out, Sr isotopic ratios found in plants and the animals feeding on them are related to those of the bioavailable portion of the soil-derived strontium.

The use of the methodology described above seems to be promising in the characterization of meat according to its geographical origin, although it has limitations that can be overcome using a combination of appropriate parameters.[5,19] Furthermore, the suitability of elements and isotopes as markers of the geographical origin should be evaluated by correlating its fingerprint in soil and water from the studied region with that of food produced in the same area. This last approach is not so common in the current literature, where we can find reports using multiple isotopes analysis and trace elements to identify the geographical origin of meat without the use of corresponding water and soil analysis, for example, the review of Franke et al.[9] on multielemental analysis and oxygen isotope analysis and other related papers.[4−6,10,19−21] Franke et al.[6] tested Sr and oxygen isotope ratios for poultry meat and dried beef, only the oxygen isotope being capable of showing clear differences between countries. A combination of multi-trace elemental analysis[19] and oxygen isotope analysis[22] would seem to be appropriate for beef origin discrimination. Measurement of carbon and nitrogen isotopes has also shown potential in differentiating between beef originating from Japan, Australia, and the United States,[3] as well as beef originating from Europe and the United States.[23] Heaton et al.[5] combined the analysis of multielement isotope and trace element of beef, obtaining good results in discriminating the origin of meat from Europe, South America, and Australasia. Despite these multiple reports of using the elemental composition and isotopic pattern to assess the geographical origin of meat, there are only a few papers considering the association with soil and water from places where cattle grew.[3,24]

Our main goal was to obtain a reliable fingerprint to assess the origin of Argentinean meat, considering both elemental composition and isotopic pattern from meat as well as investigating the link between meat composition and that of soil and drinking water geographically related.

MATERIALS AND METHODS

Reagents and Materials. Ultrapure water (<5 μg L^{-1} TOC) was obtained from a purification system Arium 61316-RO plus Arium 611 UV (Sartorius, Germany). Inductively coupled plasma multielement standard solution Merck VI CertiPUR was obtained from Merck Química Argentina (Buenos Aires, Argentina). The composition and concentration of the Merck VI standard was as described in the accompanying certificate of analysis provided by the manufacturer. Nitric acid (63.7%), sub-boiling grade, was prepared from analytical grade acid using a distiller (Figmay Sub-boiling distiller, Córdoba, Argentina). The purity of nitric acid was verified by ICP-MS. Filters (0.45 μm, HAWG04756) were obtained from Millipore (São Paulo, Brazil). Trace element certified reference material (CRM) 8414 (bovine

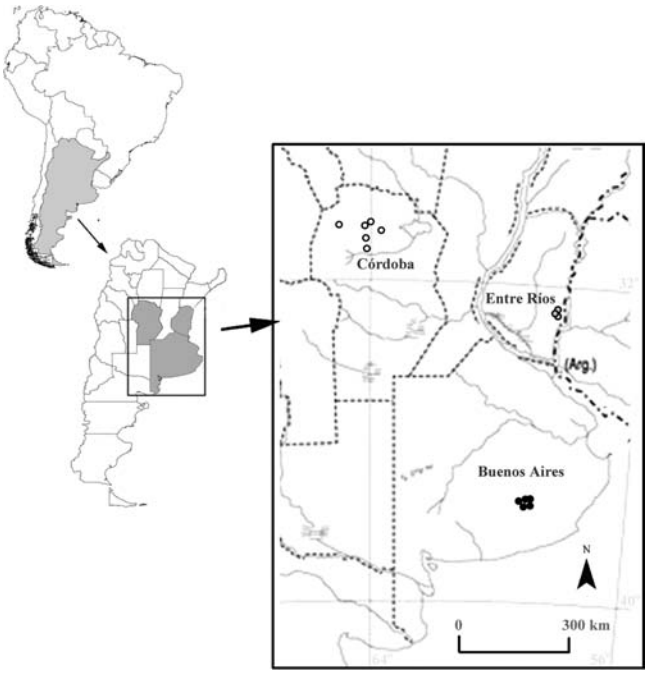

Figure 1. Map of three regions where soil, water, and beef samples were collected: (open circles) farms located in Córdoba; (gray circles) farms located in Entre Ríos; (black circles) farms located in Buenos Aires.

muscle powder) was obtained from the National Institute of Standards and Technology (Gaithersburg, MD). The best estimate values for constituent elements in CRM 8414 are detailed in the accompanying certificate of analysis provided by the manufacturer. All other reagents were of analytical grade.

Sampling. Soil, drinking water, and meat samples were collected from three regions of Argentina: Buenos Aires, Córdoba, and Entre Ríos, which are considered to be the main beef-producing areas in the country (Figure 1). The sampling area in the Province of Buenos Aires is located between 37° 21′ and 37° 28′ south latitude and between 59° 09′ and 59° 14′ west longitude; the Córdoba area is located between 30° 75′ and 31° 10′ south latitude and between 63° 64′ and 64° 90′ west longitude, whereas the Entre Ríos sampling area is located between 32° 46′ and 32° 54′ south latitude and between 58° 30′ and 58° 40′ west longitude. The altitude varies from 460 m above sea level in Córdoba to 240 m in Buenos Aires to 30 m in Entre Ríos.

Soil and water samples were obtained from farms where cows were grown during the spring season (2007 and 2008). Samples (meat, soil, and water) were obtained from different farms situated in the three regions under study: six farms within the Province of Córdoba, five within the Province of Buenos Aires, and three within the Province of Entre Ríos.

Soil. Samples were collected using stainless steel shovels and were stored in individual black plastic bags (darkness). Soils were sampled at depths from 10 to 20 cm to avoid surface-soil pollution arising from the surrounding environment and to reduce the effects of fertilizers and variable organic matter content.[17] A total of 111 soil samples were analyzed (37 from each region).

Water. A total of 120 groundwater samples were obtained (40 from each region) from the same farms used for soil and meat sampling. Samples were collected using prewashed 1 L plastic bottles.

Meat. A total of 83 samples of meat were collected from small slaughterhouses to which the monitored farms sent cattle for processing. Twenty-seven samples were collected in the Province of Buenos Aires, 25 from Córdoba, and 31 from Entre Ríos. Five hundred grams of neck muscle was obtained 24 h after slaughter. Visible fat was trimmed off with a ceramic knife before the samples were lyophilized.

Elemental Analyses. *Sample Preparation. (a) Bioavailable Soil Fraction.* Samples were dried at 40 °C during 2 days. Afterward, soils were homogenized and sieved through a 2 mm acrylic sieve, followed by further drying at 40 °C overnight. Twenty grams of dried sieved soil was weighed into an erlenmeyer flask with 50 mL of 1 M NH_4NO_3. The resulting suspension was shaken for 2 h, at room temperature. Subsequently, the suspension was allowed to settle for 1 h, filtered through 0.45 μm (HAWG04756, Millipore), and acidified with 0.5 mL of concentrated nitric acid (sub-boiling grade). Some samples were spiked to verify recovery percentages of different elements, so variable amounts of individual standard solutions (1000 mg L^{-1} in 1% nitric acid) were added to 40 g of dried sieved soil sample to double the starting concentration for each element. The rest of the procedure was the same as used for nonspiked samples. All recoveries were between 80 and 120%. All samples were prepared in duplicate.

(b) Water. Samples were acidified with ultrapure nitric acid and filtered using 0.45 μm (HAWG04756, Millipore) filters.

(c) Meat. Samples were mineralized using a microwave oven (Anton Paar 3000, Vienna, Austria). Briefly, freeze-died beef tissues (0.5 g) were introduced in quartz vessels, followed by the addition of a mixture of 7 mL of ultrapure concentrated nitric acid and 5 mL of ultrapure hydrogen peroxide solution (31%); vessels were kept open until no fumes were observed (2−3 h). Afterward, vessels were cap-closed and heated using the following power sequence: a 10 min ramp to 400 W, held for 50 min (maximum temperature = 169 °C; maximum pressure = 75 bar), followed by a final step (15 min) disabling the power to reach pressure equilibration. Mineralized samples were quantitatively transferred to 25 mL volumetric flasks, completing the volume with ultrapure water, followed by filtration using 0.45 μm filters. CRM 8414 was processed and measured in the same way used for meat.

(d) Instrumental Operation. Thirty-three elements were quantified in soil, water, and meat samples: Li, B, Na, Mg, Al, K, As, Fe, Ca, V, Mn, Co, Ni, Cu, Zn, Ga, Se, Rb, Sr, Mo, Cd, Cs, Ba, La, Ce, Nd, Sm, Eu, Yb, Lu, Tl, Pb, and U. The analysis was carried out by using a quadrupole inductive plasma mass spectrometer (Q-ICPMS) for all elements except sodium. A Thermo-Elemental X7 series (Thermo Fisher Scientific, Bremen, Germany), equipped with an ASX-100 autosampler model (CETAC Technologies, Omaha, NE), was used. The sample introduction system consisted of a microflow concentric nebulizer, Peltier cooled spray chamber, and 1.5 mm i.d. fixed injector torch. The RF forward power was 1350 W for all experiments, and the interface was fitted with Ni sampling and skimmer cones designed for low polyatomics formation. Two operation modes were used: with and without collision cell technology (CCT). CCT mode measurements were performed for Mg, K, Ca, V, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Rb, Sr Mo, Cd, Ba, Pb, and U. The collision cell was flushed with 7% H_2 in a He high-purity mixture. The elements Li, B, Al, Cs, La, Ce, Nd, Sm, Eu, Yb, Lu, and Tl were measured without operating the collision cell with gas, and thus full sensitivity was obtained. The oxide ratio and double-charged species were maintained below 1% for both modes of operation. All of the Q-ICPMS measurements were performed using Sc, In, and Re as internal standards. Sodium measurements were carried out by flame atomic absorption spectrometry (FAAS) using a Perkin-Elmer 3030 (Boston, MA) in an air−acetylene flame. All meat, water, and soil samples were diluted 10-fold using a 1% HNO_3−0.5% HCl mixture before Q-ICPMS measurements. Standards and blanks were prepared using the same mixture (1% HNO_3−0.5% HCl). Instrumental and procedural blanks were determined together with samples, and the means of five runs were obtained for each sample. Full quantitative analysis was performed against calibration standards for each element. Precision (%CV) was below 5% considering five measures on the same

Table 1. Means and Standard Deviations of Measured Elements and Isotope Ratios Corresponding to Soil, Water, and Meat According to Region of Origin[a]

	soil			water			meat		
variable	Buenos Aires	Córdoba	Entre Ríos	Buenos Aires	Córdoba	Entre Ríos	Buenos Aires	Córdoba	Entre Ríos
Al	129 ± 105 b	59 ± 78 a	95 ± 94 b	10 ± 32 a	25 ± 34 b	2 ± 2 a	5.8 ± 7.0 a	5.4 ± 5.2 a	4.5 ± 7.6 a
As	2.6 ± 1.9 a	7.8 ± 16.5 b	2.8 ± 1.3 a	ND	ND	ND	ND	ND	ND
B	337 ± 118 b	290 ± 212 b	147 ± 58 a	<LOD	<LOD	<LOD	0.10 ± 0.12 a	0.24 ± 0.25 b	0.26 ± 0.27 b
Ba	43573 ± 8541 b	20961 ± 7371 a	74608 ± 39438 c	110 ± 29 b	54 ± 43 a	212 ± 95 c	0.16 ± 0.18 a	0.21 ± 0.31 a	0.34 ± 0.21 b
Ca	2398 ± 799 a	2702 ± 3525 a	1450 ± 1796 a	74 ± 125 b	57 ± 32 a	70 ± 17 b	0.24 ± 0.19 a	0.38 ± 0.46 a	0.29 ± 0.14 a
Cd	9.8 ± 25.2 b	0.8 ± 0.6 a	1.1 ± 0.8 a	0.04 ± 0.12 a	0.01 ± 0.06 a	0.07 ± 0.15 a	<LOD	<LOD	<LOD
Ce	1.5 ± 0.8 a	1.4 ± 1.8 a	5.1 ± 3.9 b	0.10 ± 0.21 a	0.35 ± 0.64 b	0.06 ± 0.05 a	<LOD	<LOD	<LOD
Co	4.6 ± 2.6 a	3.0 ± 5.9 a	10.5 ± 8.1 b	0.10 ± 0.17 a	0.13 ± 0.18 a	0.12 ± 0.17 a	<LOD	<LOD	<LOD
Cs	39 ± 7 a	32 ± 14 a	48 ± 23 b	<LOD	<LOD	<LOD	0.11 ± 0.53 a	0.02 ± 0.01 a	0.08 ± 0.16 a
Cu	16 ± 6 a	39 ± 57 b	23 ± 10 a	16 ± 20 b	3 ± 3 a	38 ± 40 c	3.26 ± 0.36 b	2.49 ± 0.47 a	3.31 ± 0.45 b
Eu	4.5 ± 0.8 b	2.2 ± 0.9 a	8.9 ± 3.8 c	0.02 ± 0.02 b	0.01 ± 0.01 a	0.04 ± 0.02 c	<LOD	<LOD	<LOD
Fe	41 ± 40 a	52 ± 43 a	41 ± 19 a	<LOD	<LOD	<LOD	ND	ND	ND
Ga	0.06 ± 0.06 a	0.08 ± 0.10 a	0.45 ± 0.35 b	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
K	905 ± 327 b	1064 ± 1422 b	364 ± 205 a	8.1 ± 2.3 b	8.1 ± 4.1 b	3.6 ± 2.0 a	13.3 ± 0.8 a	13.4 ± 0.8 a	13.4 ± 1 a
La	6 ± 8 a	37 ± 136 a	16 ± 58 a	0.41 ± 0.43 b	1.10 ± 1.90 a	0.17 ± 0.22 b	<LOD	<LOD	<LOD
Li	201 ± 58 a	191 ± 80 a	205 ± 100 a	15 ± 6 a	44 ± 44 c	28 ± 10 b	0.08 ± 0.05 b	0.04 ± 0.04 a	0.07 ± 0.06 b
Lu	0.023 ± 0.007 b	0.015 ± 0.013 a	0.040 ± 0.010 c	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Mg	568 ± 62 b	516 ± 117 a	611 ± 78 c	37 ± 5 c	16 ± 11 b	12 ± 3 a	0.81 ± 0.05 a	0.81 ± 0.06 a	0.82 ± 0.06 a
Mn	3692 ± 2278 b	1408 ± 3894 a	8474 ± 6567 c	3.4 ± 5.9 a	9.2 ± 14.3 a	5.9 ± 13.4 a	0.47 ± 0.08 b	0.37 ± 0.08 a	0.50 ± 0.08 b
Mo	1 ± 1 a	17 ± 84 a	1.2 ± 1.3 a	<LOD	<LOD	<LOD	0.08 ± 0.04 a	0.09 ± 0.08 a	0.08 ± 0.06 a
Na	0.022 ± 0.038 a	0.064 ± 0.100 a	0.043 ± 0.093 a	40 ± 19 a	122 ± 102 b	103 ± 45 b	2272 ± 335 a	2592 ± 479 b	3135 ± 326 c
Nd	1.27 ± 0.56 a	0.98 ± 1.37 a	2.40 ± 1.30 b	0.070 ± 0.250 a	0.200 ± 0.340 b	0.001 ± 0.003 a	<LOD	<LOD	<LOD
Ni	13 ± 8 a	8 ± 12 a	40 ± 28 b	0.17 ± 0.49 a	0.26 ± 0.64 a	0.29 ± 0.88 a	0.04 ± 0.06 a	0.01 ± 0.03 a	0.03 ± 0.05 a
Pb	0.36 ± 0.79 a	0.38 ± 0.57 a	1.30 ± 1.40 b	0.16 ± 0.25 a	0.42 ± 0.45 a	1.40 ± 1.90 b	<LOD	<LOD	<LOD
Rb	1294 ± 218 b	911 ± 518 a	1488 ± 401 c	<LOD	<LOD	<LOD	3.8 ± 0.7 a	5.1 ± 1.5 a	18.1 ± 4.2 b
Se	2.3 ± 2.5 a	4.9 ± 3.6 b	3.6 ± 2.4 b	<LOD	<LOD	<LOD	0.19 ± 0.06 a	0.45 ± 0.10 c	0.35 ± 0.22 b
Sm	0.33 ± 0.10 a	0.24 ± 0.27 a	0.60 ± 0.19 b	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Sr	18407 ± 4893 a	17287 ± 8654 a	29348 ± 12078 b	746 ± 124 a	781 ± 869 a	699 ± 211 a	0.35 ± 0.27 a	0.52 ± 0.58 a	0.73 ± 0.39 b
Tl	12.5 ± 4.2 b	4.9 ± 3.3 a	8.8 ± 3.3 c	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
U	5.0 ± 4.6 b	0.7 ± 2.8 a	0.3 ± 0.5 a	6.1 ± 6.5 a	41 ± 63 b	12.5 ± 7.6 a	<LOD	<LOD	<LOD
V	7.5 ± 6.8 b	4.0 ± 8.0 a	9.8 ± 7.9 a	63 ± 18 b	40 ± 53 a	36 ± 12 a	<LOD	<LOD	<LOD
Yb	0.11 ± 0.04 a	0.08 ± 0.08 a	0.15 ± 0.06 b	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Zn	64 ± 35 b	32 ± 25 a	96 ± 56 c	134 ± 236 a	45 ± 97 a	405 ± 737 b	210 ± 35 b	176 ± 39 a	215 ± 21 b
Ca/Sr	137 ± 51 b	171 ± 232 b	51 ± 50 a	<LOD	<LOD	<LOD	800 ± 348 b	759 ± 246 b	413 ± 60 a
K/Rb	711 ± 241 b	1092 ± 587 c	260 ± 147 a	101 ± 18 a	105 ± 71 a	103 ± 15 a	3659 ± 724 c	2827 ± 724 b	787 ± 214 a
$^{87}Sr/^{86}Sr$	0.7066 ± 0.0004 a	0.7100 ± 0.003 c	0.7076 ± 0.0002 b	0.7072 ± 0.0002 a	0.7133 ± 0.0020 c	0.7078 ± 0.0004 b	0.712 ± 0.002 a	0.718 ± 0.020 b	0.712 ± 0.004 a
$\delta^{13}C$	−24.14 ± 0.92 a	−19.82 ± 1.59 b	−20.02 ± 0.92 b	ND	ND	ND	−19.81 ± 8.89 a	−13.86 ± 1.00 b	−20.02 ± 1.76 a
$\delta^{15}N$	7.7 ± 0.9 b	7.0 ± 1.4 a	9.0 ± 1.3 c	ND	ND	ND	8.10 ± 0.61 b	6.72 ± 0.52 a	9.55 ± 0.72 c

[a] ND, not determined; <LOD, below limit of detection. Element values are reported in $\mu g\ kg^{-1}$ except for Na, K, Ca, and Mn, which are in $mg\ kg^{-1}$. Isotopes ratios are expressed in δ units (‰, per mil). IDL ($\mu g\ L^{-1}$): B (0.2), Cd (0.3), Ce (0.08), Co (1), Cs (0.01), Eu (0.01), Fe (0.1), Ga (0.5), La (0.1), Lu (0.004), Mo (0.2), Nd (0.03), Pb (0.2), Rb (0.1), Se (1), Sm (0.01), Tl (0.06), U (0.01), V (3), Yb (0.01). Different letters in the same row for a matrix indicate significant differences $p < 0.05$.

sample. Instrument detection limits (IDL) are reported in Table 1. All samples were analyzed in duplicate.

Strontium Isotope Analysis. *Sample Preparation. (a) Soils: Bioavailable Fraction.* Fifty milliliters of bioavailable extracted solution, obtained as described for elemental analysis, was evaporated to dryness and redissolved in 1 M nitric acid (HNO_3). After solution, Sr was separated by ion exchange chromatography.

(b) Bulk Soil. Before digestion, ~100 mg of the sieved, dried bulk soil samples was placed in porcelain crucibles, introduced in a high-temperature muffle furnace, and ashed at 550/600 °C during 12 h to eliminate the organic compounds. Afterward, ~50 mg of the resulting material was digested in a mixture of concentrated HNO_3 and concentrated HF acid in a Savillex digestion vessel and heated overnight in an oven at ~120 °C. The resulting solution was evaporated on a hot

plate; the remaining material was redissolved in 6 N HCl and again evaporated to dryness on a hot plate. The dry material was redissolved three times in 8 M HNO_3, evaporating to dryness each time. The remaining product was dissolved in 1 M HNO_3 and loaded onto the ion exchange chromatography column.

(c) Water. Water (250−300 mL) was partially evaporated in a flask using a heating mantle. When the volume was reduced to 10 mL, the water was transferred to a Savillex Teflon vial and evaporated to dryness under an IR lamp. The residue was redissolved in 1 M nitric acid (HNO_3) and loaded onto the column to separate Sr by ion exchange chromatography.

(d) Beef. Beef samples were processed according to a dry-ashing technique. An aliquot of 5 g of dry sample was placed in porcelain crucibles in a high-temperature muffle and ashed at 550/600 °C, during 18−20 h. After cooling, the residues were treated with concentrated nitric acid on a hot plate. After that, all of the samples were transferred to the muffle furnace during 18 h in the same conditions as before. The white ashes obtained were dissolved in 1 M nitric acid and loaded onto the ion exchange chromatography column.

For Sr separation techniques the ion exchange chromatography columns were disposable and made from 5 mL pipet tips with a precleaned porous frit. Sr Spec resin (Eichrom Technologies) was used. Nitric acid of different concentrations was used as eluent. Merck Suprapur nitric and fluorhydric acid and ultrapure water (Barnstead E-Pure Water System) were used.

$^{87}Sr/^{86}Sr$ Ratio Analysis. The strontium isotope ratios were measured in a thermal ionization mass spectrometer (TIMS). The measurements were carried out using a double-filament rhenium ion source. NBS SRM 987 was employed as standard to determine the instrumental bias in each set of analyses, and the Eimer & Amend Standard (Massachusetts Institute of Technology) was also regularly analyzed to check for proper operation of the mass spectrometer. Measured ratios were corrected for mass fractionation using $^{88}Sr/^{86}Sr = 8.375209$. ^{85}Rb was monitored in each block of data to quantify any interferences of ^{87}Rb.

Stable Isotopes. Natural abundance stable isotope ratios, $\delta^{13}C$ and $\delta^{15}N$, were measured in bulk soil and defatted beef tissue samples.

Sample Preparation. (a) Soil. The soil samples were dried in an oven at 40 °C until constant weight and crushed in a porcelain mortar. Aggregates were sieved using a stainless steel sieve (2 mm mesh). A representative portion of the soil (10 g) was obtained by quartering and was ground in a mill to obtain a fine powder (particle size < 40 μm). This subsample was stored until $\delta^{15}N$ determination.

(b) Meat. The lipid component of freeze-dried beef tissues was extracted in a Soxhlet apparatus with hexane. The dry beef sample was placed in an extraction thimble, and the fat was extracted during 6 h.[8] The defatted beef mass was dried in a fume hood overnight until constant weight. The fatfree beef muscle was stored at 4−8 °C in screw-capped recipients until analysis.

Pulverized soil and defatted beef muscle samples were weighed into tin capsules for dual C and N analysis.

Isotopic Analysis. After the sample had been loaded into the tin capsule, it was sealed and dropped into the reaction tube of a Carlo Erba Elemental Analyzer. After the combustion, gases were separated by a gas chromatographic (GC) column. The GC effluent was transferred to the stable isotope ratio mass spectrometer (Thermo Fisher DELTA V Plus) using a ConFlo IV interface. The isotope ratios were normalized using laboratory working standards, calibrated with international standards supplied by the International Atomic Energy Agency. Samples were measured in duplicate. Isotope data were expressed using the international delta notation (δ, ‰) and are referred to Vienna Pee Dee Belimnite (V-PDB) for $\delta^{13}C$ and to air-N_2 for $\delta^{15}N$. Helium was used as carrier gas. The uncertainty of the carbon and nitrogen isotopic determinations was ±0.2‰.

Statistical Analysis. Multivariate statistical methods were applied to data sets: linear discriminant analysis (LDA), generalized procrustes analysis (GPA), and canonical correlation analysis (CCA). Concentrations of elements and isotopic ratios were used as chemical descriptors for meat, water, and soil samples, and LDA in stepwise mode was performed. LDA was carried out to evaluate whether meat, soil, and water samples could be mathematically distinguished according to its geographical origin. Selection of the most significant variables was performed by forward stepwise analysis according to F value. The robustness of the classification model was evaluated by a cross-validation test, using the "leave-one-out" procedure. GPA and CCA were applied for assessing the relationship between meat and geology data (soil and water). Specifically, GPA constructs the consensus configuration of a group of data sets by applying transforms in an attempt to superimpose them. In this work, we used the Gower algorithm that minimizes within-sample variance by applying translation, scaling, and rotation to generate a p-dimensional average configuration $\boldsymbol{Y}c$. Following this, a q-dimensional group average space ($q \leq p$) is constructed from $\boldsymbol{Y}c$ by principal component analysis (PCA).[25] Therefore, GPA theory and algorithms can be applied to match meat elemental and isotopic data to the corresponding soil and drinking water data. On the other hand, CCA allowed the evaluation of the relationship between soil, water, and meat studied during this work.

Analysis of variance (ANOVA) was performed with each variable and, in the case of significance ($p < 0.05$), a DGC[26] comparison test was performed to reveal paired differences between the means.

Standardized values have been used in different figures. This approach implies standardizing raw (measured) values to mean 0 and variance 1; thus, std score = (raw score − mean)/std deviation. Standard scores allow working with the same magnitude for samples having different raw values. Application of standardized scores resulted in a clearer representation of similarity and/or difference for a given parameter in three studied matrices (soil, water, and meat).

The statistical package STATISTICA 7.1 from StatSoft Inc. (2005) and Infostat[26] was used for all statistical analyses.

RESULTS AND DISCUSSION

Characterization of Sampling Area. *Analysis of Soil Composition.* Descriptive statistics (mean and standard deviation) for measured soils are reported in Table 1. In addition to the 31 elements evaluated, we included values for K/Rb and Ca/Sr ratios. The K/Rb ratio can greatly differ among various rocks and soils.[27] On the other hand, Ca/Sr has been used mainly as a chemical tracer in geochemistry, hydrogeochemistry, and bioavailability studies.[28,29]

It can be seen that the composition of inorganic components shows differences among the three sampling areas. Concentrations of Ce, Co, Cs, Ga, Nd, Ni, Pb, Sm, Sr, and Yb were highest in Entre Ríos and similar in soils from Córdoba and Buenos Aires. On the other hand, levels of Ba, Eu, Lu, Mg, Mn, and Zn were lower in soils from Córdoba, whereas soils from Entre Ríos presented the highest content, with intermediate values in Buenos Aires. On the other hand, Ca, Fe, La, Li, Mo, and Na did not show significant differences between three studied regions. It must be noted that the concentrations of elements observed in the soils of the three studied regions fell within typical contents of uncontaminated soils.[30]

$^{87}Sr/^{86}Sr$, $\delta^{13}C$, and $\delta^{15}N$ show different variation patterns. The $^{87}Sr/^{86}Sr$ ratio presents highest values in Córdoba, followed by Entre Ríos and Buenos Aires. $\delta^{15}N$ presents highest values in Entre Ríos, followed by Buenos Aires and Córdoba. Entre Ríos

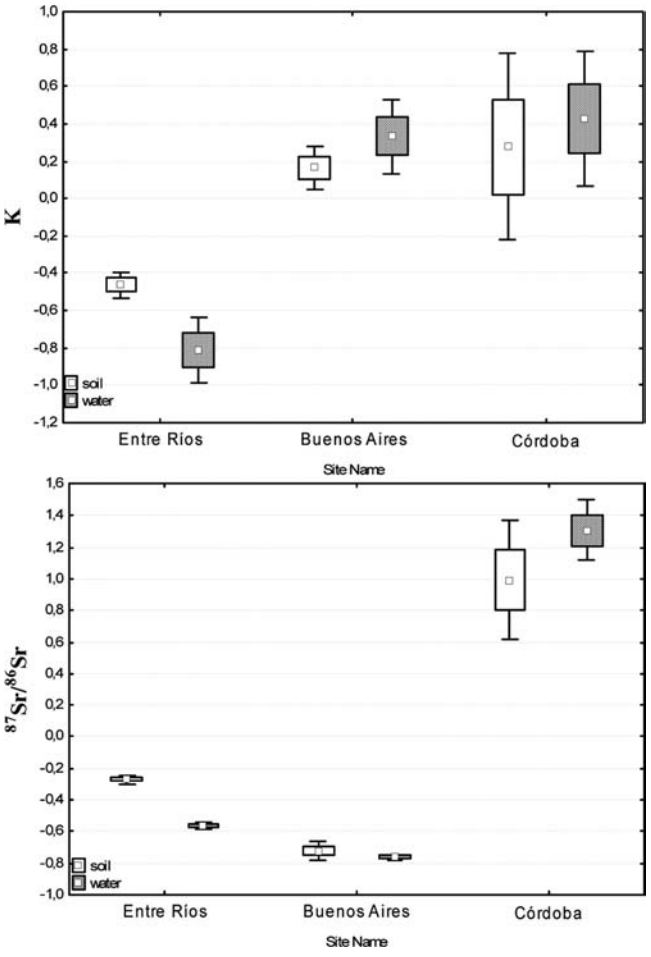

Figure 2. Standardized values of the levels of selected parameters differentiating soil and water samples. Box, mean ± SE; whisker, mean ± 1.96SE.

and Córdoba presented similar values of $\delta^{13}C$, whereas Buenos Aires presented lower values.

Stepwise DA of the soil data set allows distinguishing among three studied regions with 100% certainty, pointing out 13 variables: Al, K, Ca, Mg, Mn, Zn, Mo, Ba, Eu, Tl, $^{87}Sr/^{86}Sr$, $\delta^{13}C$, and $\delta^{15}N$ (see the Supporting Information). Some of these selected variables were also pointed out by Di Paola et al.[13] as contributing to the differentiation of wine-producing regions of Argentina, demonstrating the usefulness of the analysis of trace elements and isotopes in the chemical characterization of soil. Figure 2 shows K and the $^{87}Sr/^{86}Sr$ ratio among three studied regions (standardized values). $^{87}Sr/^{86}Sr$ ratios are capable of differentiating the three studied areas, whereas K distinguishes Entre Ríos from Buenos Aires and Córdoba.

Analysis of Water Composition. Descriptive statistics (mean and standard deviation) for measured elements and isotopes in drinking water according to the three regions of origin are also reported in Table 1. Cd, Co, Mn, Ni, and Sr did not show differences between different regions. Samples from Córdoba presented higher values of Al, Ce, La, Nd, and U, whereas Buenos Aires and Entre Ríos presented lower (and similar between them) values for these elements. Conversely, Ba, Cu, Eu, Li, and Mg presented distinctive values between the three studied regions.

$^{87}Sr/^{86}Sr$ presented significant differences between the studied areas. Samples from Córdoba presented the highest values for Sr isotope ratios, whereas Entre Ríos had intermediate values and Buenos Aires the lowest.

Stepwise DA of the water data set allows distinguishing among three studied regions with 100% certainty on the basis of six variables: $^{87}Sr/^{86}Sr$, Mg, Li, Ba, Na, and K (see the Supporting Information). Figure 2 shows K and $^{87}Sr/^{86}Sr$ variations among the three studied regions (standardized values).

Four of six selected variables ($^{87}Sr/^{86}Sr$, K, Mg, and Ba) were also pointed out by stepwise DA for discriminating soils samples. The mean values for $^{87}Sr/^{86}Sr$ and K presented the same pattern in the three regions for water and soil samples (Figure 2).

So far, soils and drinking water samples where cattle grew are clearly different at the three areas considered in this study. Taking into account the influence of geographic region in trace elements and isotopic pattern of meat and dairy products,[1,9] our next objective was to discriminate beef according to its geographical origin.

Analysis of Meat Composition. Multielement Analysis. The average mean values and the standard deviations of studied elements for meat from the three studied regions are presented in Table 1. Fourteen of 33 elements analyzed fell below the detection level in meat. An ANOVA test showed that 12 elements were significantly different at least at one region, showing that each region had meat with a typical elemental composition. Al, Ca, Cs, K, Mg, Mo, and Ni did not show significant differences between the three studied areas. In contrast, Cu, Li, M, and Zn presented lowest values in Córdoba, whereas Entre Ríos and Buenos Aires had similar higher values. Additionally, Ba, Rb, and Sr presented highest values in beef from Entre Ríos, whereas Buenos Aires and Córdoba presented similar lower values. Conversely, the Ca/Sr ratio was the lowest for meat from Entre Ríos, whereas Buenos Aires and Córdoba showed similar higher values for this parameter. Moreover, meat samples from three different origins presented significant differences in the content of Na, Se, and the K/Rb ratio.

Stable Isotopes. $\delta^{13}C$. It is well established that differences in $\delta^{13}C$ of meat and some dairy products are derived from the portions of C3 and C4 plant materials in the diet of cattle.[1,5] It has been demonstrated that herbivorous feeding with C4 plants (e.g., maize) causes higher values in beef than feeding with C3 plants.[1,5] Therefore, the observed differences in $\delta^{13}C$ value in beef from the three studied regions reflect mainly the cattle diet. For diets of pure hay and grass, the $\delta^{13}C$ value lies in the range between −26 and −30‰.[31] So far, low $\delta^{13}C$ values observed in Buenos Aires and Entre Ríos (−19.81 and −20.02‰ respectively) could be explained considering that cattle are predominantly reared in pasture dominated by C3 plants, although some maize could also be used in the diet for supplementation during the winter period. On the other hand, high $\delta^{13}C$ values observed in samples from Córdoba may indicate a high proportion of C4 plants in the diet. As stated by Boner et al.,[4] values of $\delta^{13}C$ around −14‰ are indicative that maize was the main component of the diet. This last result is also reasonable considering that the sampling area of Córdoba belongs to a semiarid region, where natural or cultivated grass is affected by drought, leading to the use of maize-supplemented feed to enable cattle growth at this area.

$\delta^{13}C$ values obtained in this work for meat sampled in Buenos Aires and Entre Ríos are in agreement with those published by Heaton et al.,[5] although these authors did not indicate from

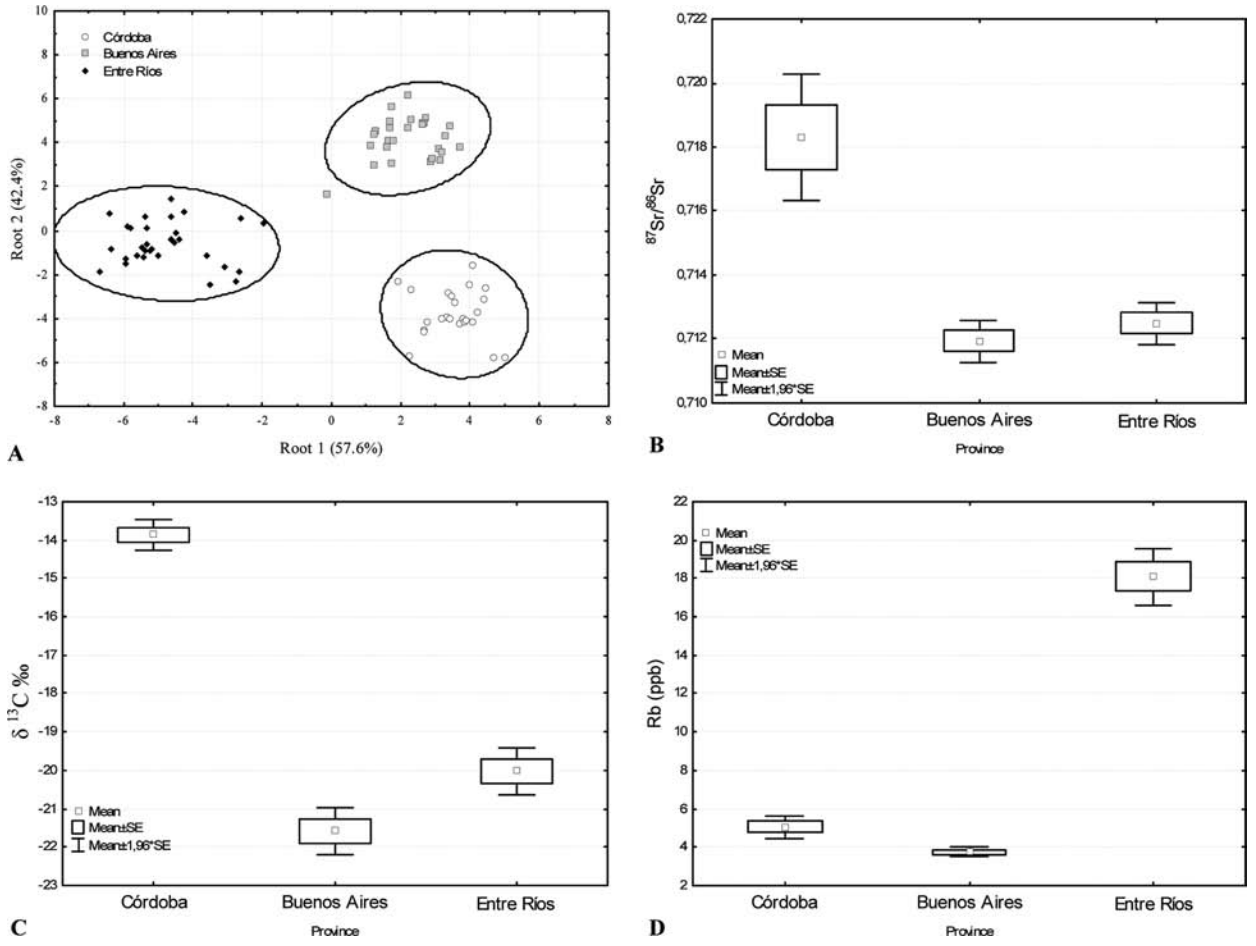

Figure 3. Discriminant analysis (canonical roots) and some selected parameters differentiating beef.

which region of Argentina their samples originated. Therefore, when applying $\delta^{13}C$ to country characterization, one should take into account differences in agricultural practices between regions to avoid misinterpretation of results.

$\delta^{15}N$. $\delta^{15}N$ values in animals depend on their diet and region of origin.[21,31,32] $\delta^{15}N$ in soil is influenced by many factors such as agricultural practices (intensive or extensive cultivation)[33] and climatic and geographical conditions.[5] Other factors include water stress[5] and the pedoclimatic conditions of the location (characteristics of the soil, altitude, and humidity), which affect the biological turnover of nitrogen, causing a higher or lower number of processes in the soil, accompanied by significant isotopic fractionation such as mineralization, nitrification, nitrogen assimilation, denitrification, and leaching accompanied by significant isotope fractionation and crossover.[18]

Meat from Buenos Aires and Entre Ríos showed enriched values for $\delta^{15}N$ (8.1 and 9.5‰, respectively), whereas samples from Córdoba had distinctive lower values (6.7‰). Regions growing cereal crops usually depend on chemical fertilizer, whereas regions producing pasture usually depend on organic fertilizer.[21,32] Chemical fertilizers are depleted in ^{15}N relative to organic fertilizer.[31] That could be the reason for significantly higher $\delta^{15}N$ values in cattle from Buenos Aires and Entre Ríos, which are characterized by pasture feeding (wheat "pampas") and tillage. As previously stated, samples from Córdoba belong to a semiarid area with higher production of cereals, mainly maize, which could affect the feed of cattle from this area. Camin et al.[31] found that carbon isotopes on cheeses were more dependent on the botanical origin (C3 and C4 plants) of the animal diet, whereas $\delta^{15}N$ values of grass and hay were more influenced by the geoclimatic conditions of the area. However, the agricultural practices used in Córdoba seem to influence the nitrogen isotope values more than the semiarid climatic conditions (aridity tends to increase $\delta^{15}N$ values in soils, plants, and animal products). On the other hand, $\delta^{15}N$ values obtained for samples from Córdoba are in agreement with those reported by Heaton et al.[34] for Argentinean beef, whereas $\delta^{15}N$ values from Buenos Aires and Entre Ríos are close to those published by Boner et al.[4] Therefore, as we mentioned for $\delta^{13}C$, the region of origin of samples in connection to both climatic and agricultural variables must be stated to avoid misinterpretation of data.

$^{87}Sr/^{86}Sr$. The $^{87}Sr/^{86}Sr$ ratio can also be useful to assess the origin of meat as it is dependent on the types of rocks and soils, not affected by human activity, climate, or season of production.[35] Córdoba samples presented the highest values, whereas Entre Ríos and Buenos Aires showed similar lower values. This can be explained by the distinctive substrate and soil parental materials. Volcanic ash falls constitute an important contributor to South American loess, which is the parent material of the modern cultivated soils of Argentina.[36] The Sr isotope composition of the Pampean loess is very close to that of the south central Andes volcanic rocks, suggesting that they were derived from such a source.[37] The Tandilia and Ventania ranges (Buenos Aires province) provided minor amounts of material to the soils and

loessic sediments of the surrounding areas,[36] which is in agreement with our current data. However, several studies based on grain size and mineralogy have highlighted the possibility of other sources for the Argentinean loess, as Pampean Ranges and Parana-Uruguay river basin,[38] which could be more relevant for other sites such as Entre Ríos. The higher radiogenic Sr values of Córdoba suggest the contribution of loess particles derived from sediments originated in the Pampean Ranges, the main source of which is Paleozoic granitic rock.

Geographical Discrimination of Meat by Multivariate Data Treatment. To evaluate the real efficiency of discrimination among the different origins of meat based on the multielement and stable isotopic analysis, a multivariate statistical method, namely, discriminant analysis, was applied.

The stepwise discriminant analysis pointed out 5 variables of 23 originals contributing to the discrimination, $\delta^{13}C$ being the most significant variable, followed by Rb, $\delta^{15}N$, Ca/Sr, and $^{87}Sr/^{86}Sr$ (see the Supporting Information). Application of canonical discriminant analysis allowed two different independent discriminant functions to be computed by linear combination of these variables. The combination of the two canonical variables accounted for 100% of variability (Figure 3A). The first canonical axis (root 1), where Rb was the most important variable, separated Entre Ríos samples from Buenos Aires and Córdoba, whereas the second canonical axis (root 2), mainly determined by $\delta^{13}C$, improved the discrimination between Córdoba and Buenos Aires. $^{87}Sr/^{86}Sr$ and $\delta^{13}C$ show the same pattern with higher values in Córdoba, intermediate values in Entre Ríos, and lower values in Buenos Aires (Figure 3B,C). Additionally, Rb shows higher values in Entre Ríos, whereas Córdoba and Buenos Aires show similar lower values (Figure 3D). The ratio Ca/Sr shows the highest values in Buenos Aires and Córdoba but a lower value in Entre Ríos (Table 1). Conversely, $\delta^{15}N$ presents higher values in Entre Ríos, intermediate values in Buenos Aires, and lower values in Córdoba (Table 1).

The use of neither isotopes nor trace elements allowed 100% discrimination with regard to the origin of meat. Isotopes alone allowed 93% of correct classification, whereas multielemental allowed 97% of correct classification according to geographical origin. On the contrary, the combined use of both data sets (isotopes and trace elements) enabled 100% certainty during discrimination, demonstrating the importance of the combination of variables for assessing the geographical origin of meat.

We have to point out that all isotopes analyzed in meat samples were selected by stepwise discriminant analysis as good discriminators of geographical origin. As stated by Boner et al.,[4] the specific isotopic conditions of the local "isotopic environment" create a constant detectable signature from the farming in the beef, which seems to be constant considering a period of at least 18 months. Beef is part of a nutrient chain from soil via plants to animals and humans. Within such nutrient chains enrichments are observed, mainly caused by metabolic processes. For conventional farming a long detectable signature should not be expected as the situation may be different. The use of silage is linked to the world market price and may change abruptly. With a change in the silage, the isotopic ratio could change as well, causing changes in the isotope composition that could result in a new isotopic pattern, different from the original. Nevertheless, by using a combination of three stable isotopes ($\delta^{15}N$, $\delta^{13}C$, and $^{87}Sr/^{86}Sr$) it is still possible to detect geographical origin as well as local feeding conditions and agricultural practices, as was the case with cattle from Córdoba in our study (Figure 3B,C and Table 1).

The isotopes included in this study have been also proposed to differentiate beef samples from different regions. Nakashita et al.,[3] Guo et al.,[21] and Horacek et al.[39] used carbon and nitrogen isotopes to discriminate beef from different countries and with different feeding systems. Boner et al.[4] used $\delta^{18}O$, $\delta^{2}H$, $\delta^{15}N$, $\delta^{34}S$, and $\delta^{13}C$ to discriminate samples from Germany, Argentina, and Chile and also to distinguish meat from organic and conventional farming. Although $^{87}Sr/^{86}Sr$ has been widely employed as a geochemical tracer, there are few reports of the use of Sr isotope ratios for food or beverage authentication.[6,40,41] In the case of animal products, Sr is transferred from the soil to pasture, which will feed the cow,[1] serving as an indicator of geographical origin. Franke et al.[6] used $\delta^{18}O$ and $^{87}Sr/^{86}Sr$ to discriminate the country of origin in dried beef, even though they found better results with $\delta^{18}O$ from water than using $^{87}Sr/^{86}Sr$. Our current results show good discrimination between the three studied areas using a combination of isotopic patterns and trace elements.

Franke et al.[9] stated that animals kept in regions with distinct characteristics could present different elemental profiles, which is the result of water intake and locally grown feeds, thus allowing their assignment to a site of origin. For instance, Rb is an alkaline metal, which is labile in the soil and easily transported into the plant, being a good indicator for the kind of soil and its geographical origin.[9,42] Rb has been previously used to discriminate the origin in chicken breast, dried beef, and raw meat.[5,19,20] In our case, Rb allowed good differentiation of meat from Entre Ríos (Figure 3D), showing similar values for Córdoba and Buenos Aires.

The ratio Ca/Sr is widely used in archaeology as a paleodietary indicator.[43,44] It has been also used to distinguish between fish spawning (breeding) sites and habitats for chum salmon.[45] Ca is an essential nutrient for plants, Sr and Ca can hinder each other, but Sr does not replace Ca in biochemical functions. Sr is not a micronutrient, and it is absorbed following the plant's metabolic requirements for Ca.[13] As far as we know, there are no studies that use this ratio as tracer of the geographical origin of food. In our case, Ca/Sr allowed good differentiation of meat from Entre Ríos, showing similar values for Córdoba and Buenos Aires (Table 1). Regional differences in trace element ratios are sometimes typical for one specific area. However, when animals switch between areas or a feedlot phase plays a role, the geographic signature could be disturbed.

Correlation between Soil, Drinking Water, and Meat Composition. It is known that the content of trace elements and isotopes in animals depends on various factors such as feed intake, drinking water, pollution, and soil composition, most of them dependent on geographic origin.[5,19,20] Therefore, we were interested in evaluating the association between chemical-isotopic profiles of soils and drinking waters and that of meat from the same geographical origin.

Some elements and isotopes exhibit a good correspondence between its content (or isotopic ratios) in soil, water, and meat for the three studied areas. For instance, $^{87}Sr/^{86}Sr$ shows the highest values in soil, water, and meat from Córdoba, lowest values in soil and water from Buenos Aires, and intermediate values in soil and water from Entre Ríos; however, meat from Buenos Aires and Entre Ríos did not present significant differences when using raw mean values (Table 1).

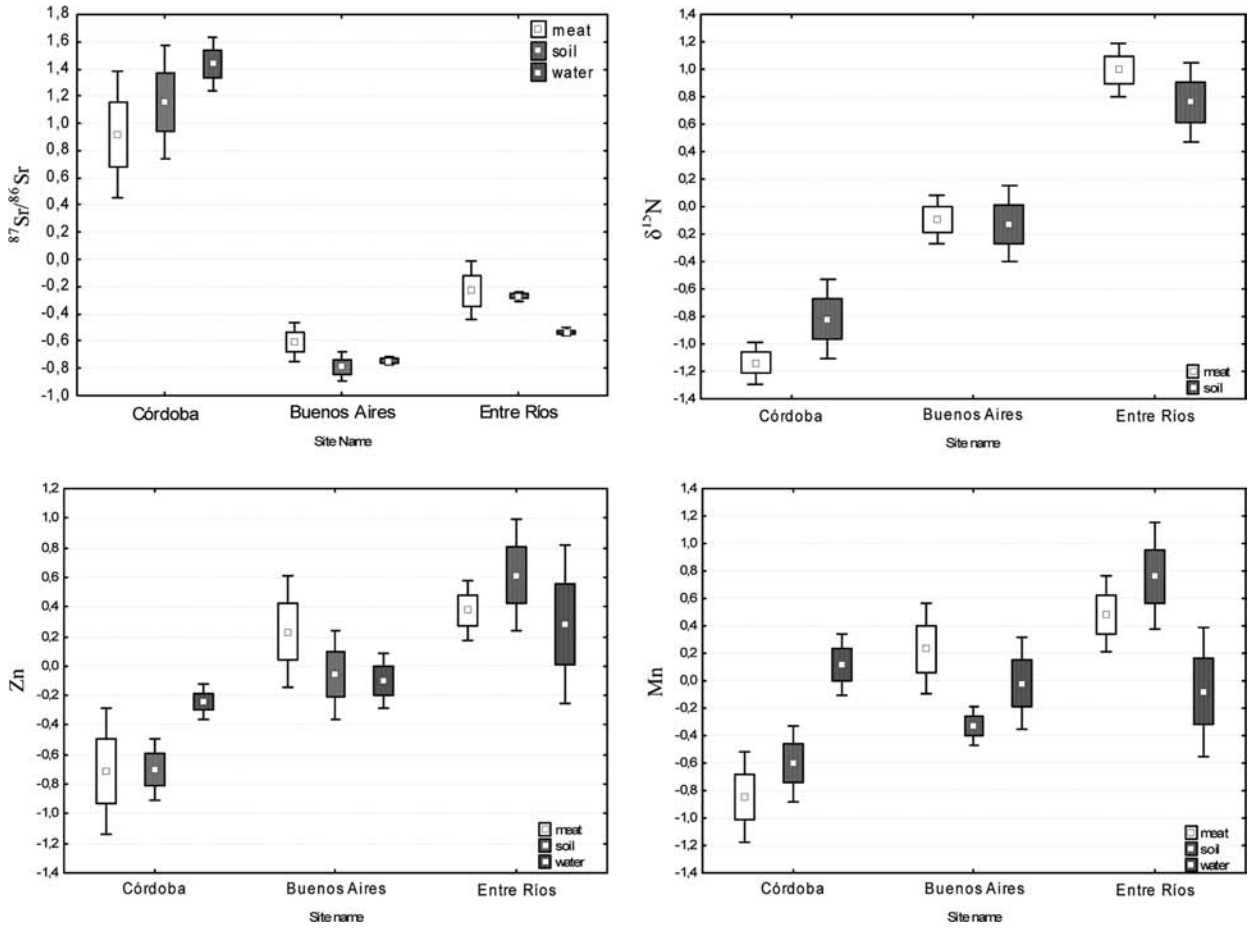

Figure 4. Correspondence between levels of several elements and isotopes in soil, water, and meat samples from different geographical areas (standardized values).

Figure 4 clearly shows that standardized values for $^{87}Sr/^{86}Sr$ and $\delta^{15}N$ allow differentiating between soil, water, and meat from three studied areas. Also Figure 4 shows that standardized values for Zn and Mn allow good differentiation between the three studied regions when considering soil and meat but not water. It is evident that standardized values for Mn are quite similar in water from the three areas. So far, it seems that isotopic composition fits quite well along the three studied matrices, whereas elemental composition fits better between soil and meat with more discrepancies with water.

Figure 5 presents standardized values for multiple elements and some isotopes measured in meat, soil, and drinking water from the three studied areas, showing the correspondence between three data sets after standardization to avoid differences arising from different magnitudes measured. From Figure 5 it is evident that many parameters have the same trend along the three studied matrices (e.g., $^{87}Sr/^{86}Sr$), whereas a few did not (e.g., Mg). Also from Figure 5 it seems that meat mostly reflects the composition from soil and, to a lesser extent, from water. However, this last assessment must be statistically demonstrated.

Looking for additional evidence on the correspondence between the three studied matrices, we decided to apply GPA. GPA produces a configuration of the different geographical regions that reflects the consensus among the three matrices (meat, soil, and drinking water). The result is a consensus alignment that uses all elements and isotopes from the three data sets. In Figure 6, the consensus configuration projected onto the plane defined by its first and second principal axes is shown, explaining 100% of variability between samples. We can observe that the three geographical origins are well separated on the basis of the elements and isotopes of soil, drinking water, and meat samples. This result shows that data obtained from meat has a significant consensus (98.9%) with those corresponding to soil and drinking water, as the three data sets project the regions in the same way onto the plane defined by its first and second principal axes. This last result gives further indication on the connection between the three studied matrices.

Finally, we applied CCA to assess the correspondence between meat and soil and drinking water composition using a more formal mathematical-statistical approach. For this purpose, four sets of variables were defined taking into account common variables between meat and soil and between meat and drinking water. The first CCA was calculated between meat and soil data sets using 22 variables (Mn, Ca, K, Mg, Cu, Ba, Sr, Na, Ni, Zn, Rb, Se, B, Al, Mo, Cs, Li, K/Rb, Ca/Sr, $\delta^{15}N$, $^{87}Sr/^{86}Sr$, and $\delta^{13}C$). This CCA showed a significant correlation ($r^2 = 0.93$, $p < 0.001$) between meat and soil. Contents of $\delta^{13}C$, $\delta^{15}N$, and K/Rb in meat as well as Ca/Sr, $\delta^{15}N$, and Mo in soil show substantial loadings on the first canonical factor; that is, they correlate highly with this factor, meaning that these variables are those that mainly contribute to the correlation between meat and soil (see the Supporting Information). The second CCA was calculated

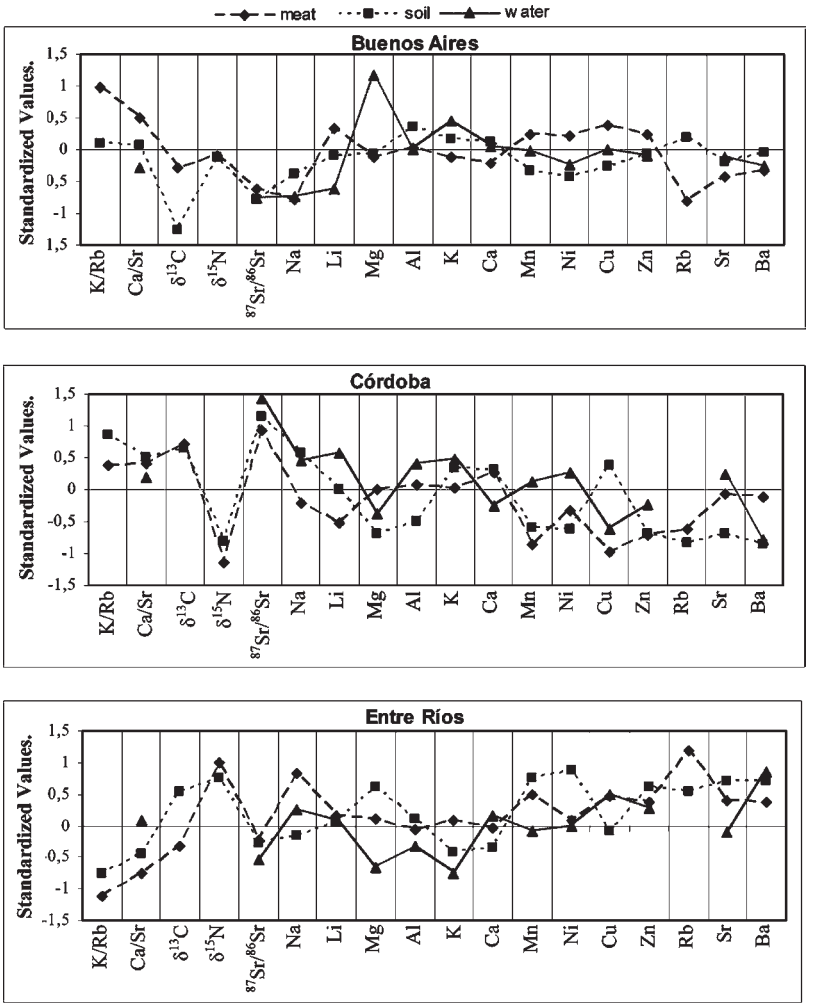

Figure 5. Standardized values for multiple elements measured in soil, water, and meat at three sampling areas.

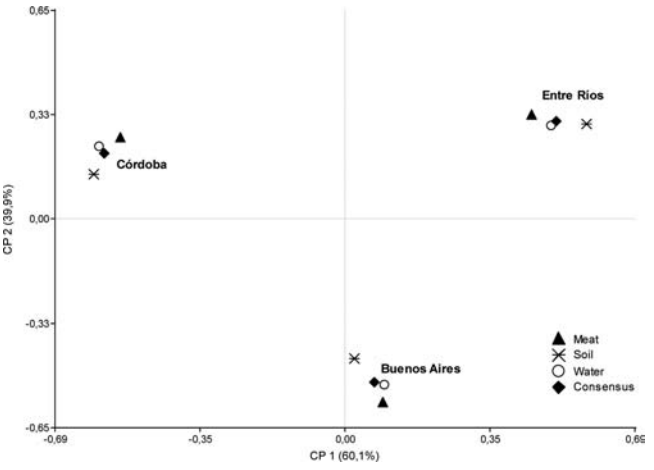

Figure 6. Consensus space from generalized procrustes analysis: plot in the plane defined by the first two dimensions.

using meat and drinking water data sets with 13 variables (Mn, Ca, K, Mg Cu, Ba, Sr, Li, Na, Ni, Zn, Ca/Sr, and $^{87}Sr/^{86}Sr$). This second CCA showed a significant correlation ($r^2 = 0.83$, $p < 0.001$) between meat and drinking water. According to the loadings on the first canonical factor Ca and $^{87}Sr/^{86}Sr$ contents in meat as well as $^{87}Sr/^{86}Sr$ content in drinking water are the variables that mainly contribute to the correlation between meat and drinking water (see the Supporting Information). Therefore, both GPA and CCA show the influence of the geology on meat composition.

Previous studies have demonstrated that the elemental composition and stable isotope fingerprint led to discrimination of the geographical origin of beef.[5] In this contribution we have found significant differences between elemental contents and isotope ratios depending on the region of origin, which allowed geographical discrimination of samples through a suitable multivariate statistical analysis.

Linear discriminant analysis enabled 100% correct classification of meat samples from different Argentinean regions when considering isotopic ratios $\delta^{13}C$, $\delta^{15}N$, and $^{87}Sr/^{86}Sr$ as well as elements such as Rb and the ratio Ca/Sr. However, the database should be increased by considering samples taken at different times to consider seasonal variations, particularly with respect to C, N, and S isotopes.

To our knowledge, this is also the first report using at least three independent statistical methods to demonstrate the correspondence between soil, water, and meat using two groups of unrelated variables. So far, we have demonstrated that meat composition is more closely related to the soil composition and,

to a lesser extent, to the water composition. Moreover, we used a data set where some parameters are associated with the geology (trace elements, Sr isotopes, $\delta^{34}S$, etc.), whereas others are related to feed characteristics (C3−C4 feeds, $\delta^{13}C$) and still others are related to agricultural practices (use of fertilizers, $\delta^{15}N$, etc.). Generalized procrustes analysis and canonical correlations were useful tools to evaluate such correspondence, which could be used when for the analysis of meat or any other food from diverse sources.

■ ASSOCIATED CONTENT

Supporting Information. Additional tables. This material is available free of charge via the Internet at http://pubs.acs.org.

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