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Data, Methods & Taxonomies

Stable Isotopes Analysis (Carbon and Nitrogen) in the Central Western Argentina Archaeology: A Review

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Abstract Stable isotopes analysis has become an essential part of the archaeological research agenda that engages ethnobiology. This paper reviews its impact in regional archaeological research focusing on the introduction of domesticated plants and their evolution in the human-environment system in Central Western Argentina (CWA). We emphasize the significance of stable isotopes in investigating the complex evolutionary history of maize adoption and agricultural practices within a dynamic Late Holocene boundary zone between farming and hunter-gathering. The 30-year history of archaeological isotopic research in CWA represents a dynamic history of old and new questions and methodologies, greater statistical sophistication and theoretical depth in its interpretation. Its impact, initially limited to reconstructing human diets, has now expanded significantly, providing powerful tools to model humans as part as a dynamic ecosystem.

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Stable Isotopes (Carbon and Nitrogen) in Central Western Argentina: An Introduction

Central Western Argentina (CWA) represents a buffer/transitional zone where lifeways vary from southern Patagonian hunter-gatherers to the northern "Andean world" of farmers (Figure 1). There were 2,000years of interactions within a shifting frontier zone between these societies with contrasting socioeconomic lifeways (Gil et al. 2020c; Lagiglia 2001). In this context, archaeological stable isotope data significantly improve the knowledge about prehistoric human diet. Focusing on this transition in human subsistence and diet, this paper summarizes how the application of stable isotopes in CWA archaeology began and its subsequent methodological and theoretical evolution.

With roots in archaeological research back into the 1970s, carbon and nitrogen isotope analysis of human tissues is a powerful tool that provides robust reconstruction of past animal and human diets that is now a widely applied methodology (DeNiro and Epstein 1978; Katzenberg 2008; Roberts 2022; Schoeninger and Moore 1992). During the 1980s, isotopic evidence detailing the antiquity of maize consumption incorporated data from human bone collagen from archaeological sites in northern South America, including the Andes (Burleigh and Brothwell 1978; van der Merwe et al. 1981). Archaeological stable isotope analysis in South America is now routinely used to examine a broad diversity of subjects such as past climate (Yanes et al. 2014; Latorre et al. 2017), paleoenvironments (Domingo et al. 2012), and human diets reconstruction at multiple spatial and temporal scales (Barberena et al. 2009; Otaola et al. 2018; Pezo-Lanfranco et al. 2024; Zangrando et al. 2013). In CWA, pioneering stable isotope research occurred during the late 1980s and early 1990s (Fernández and Panarello 1991; Fernández et al. 1999; Novellino and Guichón 1999).

Figure 1 Central West Argentina (CWA) in the macro regional context of subtropical Andes (South America), with a schematic historic boundary between hunter-gatherers and farmers (Gil et al. 2011; Lagiglia 2001; Neme et al. 2024).

In this review we begin by analyzing factors driving the variation of stable carbon and nitrogen in terrestrial ecosystems, focusing on CWA. Other elements have been studied in CWA but are not considered here (e.g., H, O, and Sr; Barberena et al. 2020; Fernández et al. 1999; Sharp et al. 2003; Ugan et al. 2012). We explore the application of stable isotope analysis to studying diet diversity in CWA focused on the introduction of domesticated plants and their evolution in the human-environment system.

Stable Isotopes in Central Western Argentina: The Natural Background

CWA is a heterogeneous environment with altitudinal variation in climate, hydrology, fauna, and vegetation. The study area (Figure 1) is between ca. 30º and 37º S latitude, including mountains in the west, plains to the east, and a volcanic area in the southeast (Figure 2). This area is a temperate, continental region with an arid to semi-arid climate. Different ecoregions include the Puna, Altoandina, Patagonia, Cardonal, and Monte deserts within the South American Arid Diagonal, where precipitation alternates seasonally between the Atlantic and Pacific anticyclones. The Puna and Altoandean desert, which comprises the cordillera above 2,500–3,000 masl, is characterized by cold, windy climatic conditions and winter predominant precipitation of 300-800 mm/year. The shrubby and herbaceous steppes of this unit are composed mostly of C_3 photosynthetic bunch grasses and shrubs (Cavagnaro 1988). The Patagonian Desert, extending south from ca. 35° S latitude, is located between 2,200 and 1,500 masl. The Patagonian semi-deserts and steppe average 200 mm/years, with a ranging of 600 to 120 mm. Most rainfall occurs during the winter. This desert is characterized by a xeric bushy steppe, and the dominant vegetation is composed of C_3 plants (Aranibar et al. 2023). The Monte desert, which covers the majority of the study area, forms a wide fringe containing the eastern plains and eastern part of the La Payunia badlands. It is characterized by an arid climate with summer dominant precipitation of 150–350 mm per year. In CWA, the most influential factor of $\delta^{13}C$ variation is the isotopic distinction between C_3 and C_4, the two dominant photosynthetic pathways. Few species exhibit another photosynthetic pathway, Crassulacean Acid Metabolism (CAM). Different factors can influence variation in nitrogen isotope values: aridity, mean annual temperatures and soil salinity. Figure 3 shows the preliminary isotopic variability in wild plants of CWA (Aranibar et al. 2023; Gil et al. 2020b). *Zea mays* is added as a comparative framework along with *Lama* sp. This provides an isotopic framework where human isotopic diet is constructed and interpreted (details in Aranibar et al. 2023; Gil et al. 2020b; Otaola et al. 2018; Peralta et al. 2022).

Stable Isotope in CWA Archaeology: Topics and Perspectives

Stable isotope research in CWA archaeology became commonplace in the 1990s (Fernández and Panarello 1991; Fernández et al. 1999; Gil 2003; Novellino and Guichón 1999) and focused on identifying the arrival and significance of maize farming. Initially, as in the rest of the Americas (Cadwallader et al. 2012), these studies assumed non-presence, or feeble signals, of wild C_4 plants in CWA ecosystems (Roberts 2022). Thus, it was presumed human tissues with high values of $\delta^{13}C$ could be interpreted as a consequence of maize consumption. If it was assumed that humans living in a predominantly C_3 plant environment have access to a C_4 cultigen that formed an essential dietary staple, then the relative importance of such a cultigen in the diet should be measurable through an isotopic

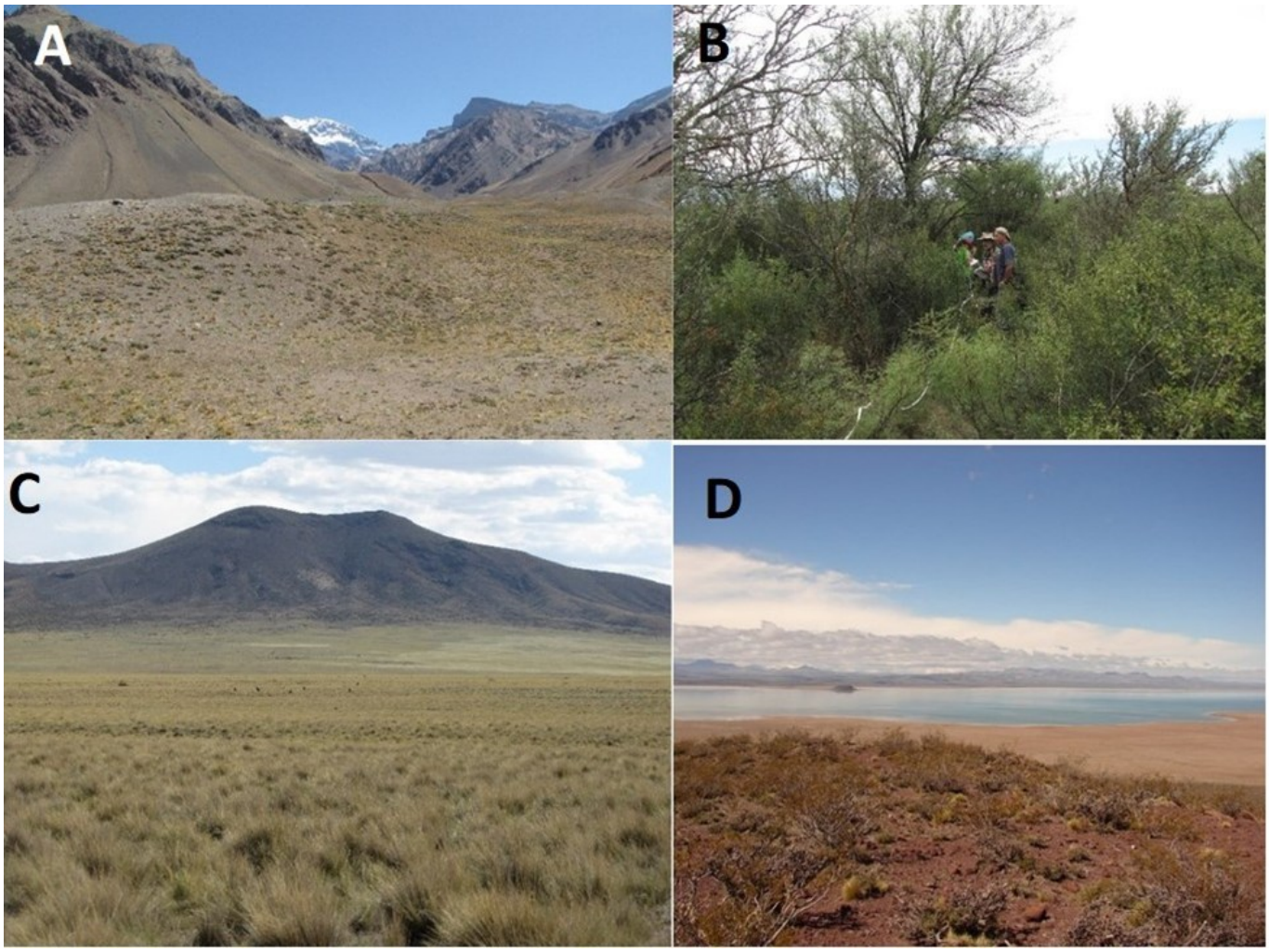

Figure 2 Environmental diversity in CWA. **A** Altoandean, **B** Monte, **C** Patagonian desert, and **D** Llancanelo wetland.

study of skeletal remains. However, this assumption should be and has been contested. Cavagnaro's (1988) paper, for instance, strongly influences the applications of stable isotopes in archaeological research by rejecting the idea of a "*wild C_3 world*" where the variation in $^{13}C/^{12}C$ can be translated in terms of the degree of maize consumption (Roberts 2022). It encourages the necessity to generate a regional baseline for understanding isotopic variation. Interpretations of diet based on isotopic data depend upon a thorough knowledge of the range and variation in isotopic compositions of foods potentially consumed.

Based on general models of plant $^{13}C/^{12}C$ and $^{15}N/^{14}N$ distribution (Ambrose 1991; Amundson et al. 2003; Kohn 2010), a baseline for altitudinal isotopic distribution model was used by Gil et al. (2016) as an initial theoretical approach. This model assumed a linear negative relationship between altitude and $\delta^{13}C$ and $\delta^{15}N$ values with decreasing altitude and increasing aridity, from the western mountains to the eastern plains. This was a baseline proposed for the tropics, with clinal altitude and climate gradients for both $\delta^{13}C$ and $\delta^{15}N$ values and was influential in the first stage of CWA isotopic research. The initial effort was to develop an isotopic baseline for dietary reconstruction focused on vertebrate fauna (Fernández et al. 2016; Gil et al. 2014; 2020a; 2024; Otaola et al. 2018). Otaola et al. (2018) presented the results of an isotopic study of $\delta^{13}C$ and $\delta^{15}N$ on bone collagen from modern samples of native wild fauna from the Monte and Patagonia deserts. However, contrary to "altitudinal" expectations, they did not observe significant differences in $\delta^{13}C$ values across different ecosystems

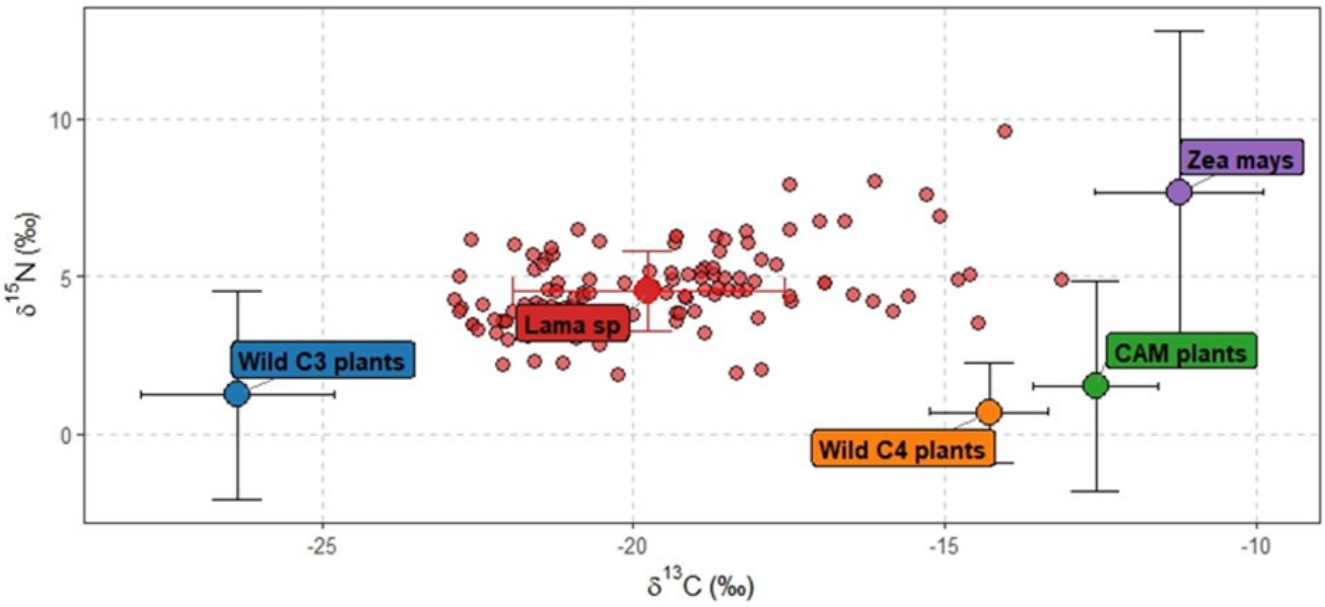

Figure 3 Isotopic variability in CWA baseline (based on Aranibar et al. 2023; Otaola et al. 2018; Peralta et al. 2022; Gil et al. 2020b).

(among Monte and Patagonia Deserts), although differences in $\delta^{15}N$ were observed.

Otaola et al. (2018) integrated the isotopic research on faunas from modern and archaeological contexts in CWA (Barberena et al. 2018; Fernández et al. 2016; Giardina et al. 2014; Gil et al. 2016, 2020b, 2024). Otaola et al. (2018) influences advance in this line of research focused on camelids (Gil et al. 2016, 2024; Gil et al. 2020a, 2023b) and rodents (Fernández et al. 2016; López et al. 2025). Otaola et al.'s (2018) regional perspective on isotopic ecology has also provided new insights into human diet and trophic relationships (Gil et al. 2020b, 2024; Gordón et al. 2018; Moscardi et al. 2022). This study demonstrates that the clinal and altitudinal model of $\delta^{13}C$ and $\delta^{15}N$ variation does not apply in CWA, highlighting a key area for research agenda. The same paper presents isotopic data for diverse vertebrate fauna in the region, enabling the development of mixed-diet models that incorporate resources beyond guanacos. Additionally, these findings contribute to refining models of past and present fauna mobility and human -animal interactions (Gil et al. 2024).

A broad isotopic plant survey is currently underway (Aranibar et al. 2023) to investigate spatial patterns of $\delta^{13}C$ and $\delta^{15}N$ of different plant types (C_3, C_4, and CAM) along environmental gradients in the eastern slopes of the southern Andes. This provides a more accurate baseline for reconstructing animal diet, mobility, and trophic relations. Aranibar et al. (2023) reject the previously presented model of increasing $\delta^{13}C$ and $\delta^{15}N$ at lower elevations and in areas of higher aridity. The spatial frequency distribution of the C_3/C_4 ratio exhibits a non-linear pattern, with higher C_4 abundances at intermediate altitudes, probably driven by atmospheric circulation patterns that generate aridity and C_4 dominance at intermediate altitudes. This pattern has a significant impact on interpreting not only the human diet and the human-fauna relationship. The modern plant baseline of isotopic variability continues to lead the exploration of new topics such as investigation of archaeological rodent remains (*Ctenomys* sp.) as a proxy of paleoclimate and/or maize cultivation in CWA (López et al. 2025).

Most applications of stable isotope analysis of human bone in CWA concern dietary reconstruction. Studies have focused on a diversity of scales from populations to individuals within archaeological sites (Gil et al. 2014, 2017; Peralta et al. 2022; Peralta and Ots 2024) and regions (Gil et al. 2006, 2017; Novellino and Guichón 1999) to summaries of diet at the regional scale (Gil et al. 2011, 2014). Recent isotopic research in archaeology includes several

studies that advance knowledge about the human past based on well-formulated archaeological questions and theoretically informed perspectives (see Roberts 2022), and research in CWA follows a similar trend (Freeman et al. 2024; Gil et al. 2024; Peralta et al. 2022, 2024). Changes in human diet are being connected with human population dynamics, and some studies consider changes in climate/ environment structure (Barberena et al. 2020; Freeman et al. 2024; Peralta et al. 2022, 2023, 2024). The focus of contemporary isotopic research is not only reconstructing the human diet, but is incorporating studies of diet as a variable to explain more theoretically oriented research (Freeman et al. 2024; Gordón et al. 2018; Peralta et al 2020). At the same time stable isotopes are advancing to connect the human-nature systems with an ecological framework (Gil et al. 2020c).

Stable Isotopes, Human Strategies, Maize and the Limits of Farming in CWA

CWA is considered the pre-Hispanic southern limit of maize agriculture in the Americas (Figure 1; Lagiglia 2001). CWA is a heuristic cultural geographic unit that includes northern Patagonia, which represents the northern limit of pre-Hispanic hunting and gathering societies; and the Northwest where the last evidence of farming was recorded (Figure 1). Thus, CWA is a border area between hunting and gathering to the south and farming to the north and, consequently, the archaeological record is a mosaic of these diverse human strategies (Figure 1). The presence of cultivars such as maize, squash, bean, and quinoa (Lagiglia 2001) as well as ceramic technology have been interpreted as evidence of farming and sedentary settlement in the region (Lagiglia 2001). In some cases, it has been associated with pastoralism as well. Maize is the most ubiquitous domesticate in pre-Hispanic CWA and dates back to as early as ca. 2000 years ago (Gil, 2003). It is generally assumed that maize was a significant resource when added to the central western human diet 2,000 years ago and that it rapidly became a staple until historic times.

CWA subsistence adaptations varied along a northern to southern gradient during the historic period, when Spanish chronicles described the ethnics groups. To the north of Diamante River Valley, sedentary or semi-sedentary Huarpe horticulturalists occupied a vast plain area, about 35°–30° S, while to the south, Puelche and Pehuenche remained with a hunter-gatherer lifestyle from Diamante River to the province of Neuquén, about 35°-40° S. Early archaeological research projected this cultural-geographic pattern back ca. 2,000 years, associating its origin with the arrival of maize agriculture in the region (Lagiglia 2001).

As stated above, early studies in the archaeological application of isotopic analysis in CWA focused on the origin and significance of maize consumption in the region (Gil 2003; Novellino and Guichón 1999). Since then, stable isotopic analysis in CWA archaeology has rejected previous ideas and generated new insights about the initial incorporation of domesticate resources, their spread, and time/space variation. In its early use, the analyses were based on $\delta^{13}C$ on bone collagen (Gil 2003; Novellino and Guichón 1999). These studies used a heuristic threshold to define the degree of maize consumption: $^{13}C/^{12}C > -14‰$ indicates a diet with >50% of maize on diet (Tykot 2006). It became a "maize rule" for determining the presence of maize as a resource in CWA human diet. The advance of research added the $\delta^{15}N$ as another proxy to monitor the variability in human diet (Gil et al. 2006). With this second element, the research utilizes an analytical perspective with bi-plot ($\delta^{13}C$ and $\delta^{15}N$) including human bone collagen, plant and animal resource values to build a visual comparison (Gil et al. 2006, 2011). The incorporation of $\delta^{15}N$ allowed estimating trophic positions (Freeman et al. 2024; Peralta et al. 2022) and includes aspect of climate (López et al. 2025). Additionally, new research also incorporated the analysis of stable isotopes in bone carbonate (Gil et al. 2006). It allowed differentiate routing of diet component sources such as protein source-inferred from bone collagen- and total diet including proteins, lipids and carbohydrates- inferred from bone carbonate.

Two key assumptions regarding the initial adoption of agriculture and its spread in CWA have been revised through advances in stable isotope analysis of archaeological remains. First, the early assumption that agriculture abruptly emerged in CWA and became the dominant subsistence strategy over the last two millennia has been challenged. Second, the notion that this early agricultural transition established a long-standing and fixed boundary between hunter-gatherers and farmers in the region has also been reconsidered.

Gil et al. (2011) rejected the idea that maize was significant in the human diet as early as 2000 years ago

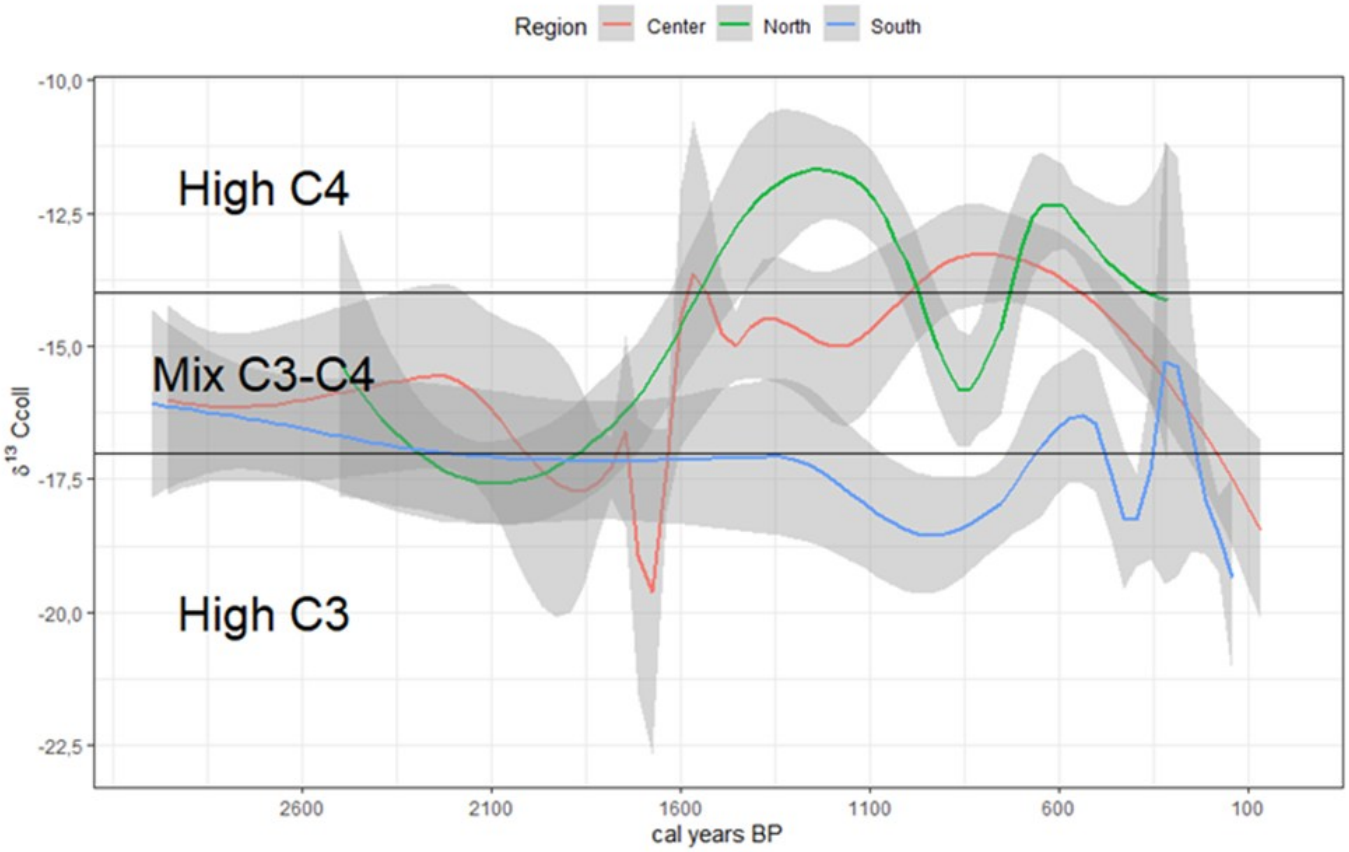

Figure 4 Bone collagen smooth trend on $\delta^{13}C$ for CWA; North (32° to 34° SL), Center (34° to 36° SL), South (36° to 38° SL). Data from Gil et al. (2011), Freeman et al. (2024), and Peralta et al. (2022).

in a study that sampled archaeological remains from northwest Mendoza where there was historic evidence of farming and contact with the Inka. These authors concluded that maize was incorporated early but only became significant in the human diet after ca. 1,000 years BP (Gil et al. 2011; Figure 4). Subsequent investigations explored the earliest maize isotopic signal and its geographic distribution improving sample size, explored greater temporal depth, and expanded geographic scale within CWA (Gil et al. 2011, 2014). $\delta^{13}C$ data indicates that C_4 resources initially (ca. 2000 years BP) were not very significant in the CWA human diet, and when they were, it was highly variable among contemporaneous individuals. However, this research shows that after 1,000 years BP, C_4 resources became a significant energy source in the northern CWA (Freeman et al. 2024; Gil et al. 2009; Peralta et al. 2022, 2024; Figure 4). The $\delta^{13}C$ and $\delta^{15}N$ values show a population with a highly heterogeneous diet. This spatial and temporal expansion in the database of stable isotopes on human bone allows us to visualize an extensive range of variation that cannot be simply assumed as a directional increase of C_4 thought time (Figure 4).

In addition to the studies described above, stable isotopes have been applied as a proxy to investigate additional factors that trigger variation in time and space of maize consumption (Barberena et al. 2020; Gil et al. 2016; Peralta et al. 2022). Gil et al. (2016) explored the impact of climate on farming production using dietary reconstruction over the last 2500 years. In northern Mendoza and San Juan provinces, maize enters the archaeological record at the beginning of this period and increases in importance through time, stabilizing at approximately 50% of the overall diet between 1000- and 600-years BP. These data indicate that the most significant increase in maize consumption predated the Inka occupation of the northern Andes, coinciding with a similar pattern in central Chile (Falabella et al. 2007). An important conclusion, therefore, is that expansion in maize

consumption was not an effect of Inka expansion. After that period of increase in the consumption of maize, the onset of cold conditions associated with the Little Ice Age induced changes in human diets in CWA, with declining stable isotope values after 600 BP. Results indicate a decrease in C_4 inputs in human collagen and, by extension, a drop in maize consumption after that time. In a related study geographically situated farther to the north in CWA, Barberena et al. (2020) proposed a migration influx into Uspallata Valley between ca. 700 to 400 BP, based on evidence from multiple data types. Their isotopic data (C and N associated with Sr) suggests that a significant fraction of the migrants came from farming communities that practiced maize agriculture, as opposed to communities incorporating a broader subsistence base previously recorded as common in the area. Recently, Peralta et al. (2022) added data on human population dynamics as another variable to understand time/space variation in maize consumption. They observed that the population dynamic of late Pleistocene-Holocene hunter-gatherers changed its trajectory toward rapid growth ca. 2,300 years BP. Peralta et al. (2022) identified several population "booms" followed by notable population "busts" in Northwest Mendoza (CWA). Stable isotope relationships of $^{13}C/^{12}C$ on human bone follow a similar trend, indicating an increase in the consumption of maize during the population boom periods and decreased consumption during the bust periods. Peralta et al. (2022) propose that the variation in human population during the last 2500 cal. years BP is associated with maize consumption. The increase in population density after the incorporation of domesticates is associated with higher investment and reliance on maize agriculture. The use of domesticated plants, maize in particular, varies over time in association with the recorded population oscillations. This dynamic association between population and maize consumption probably was due to delayed feedback between subsistence choices at a small-scale and population density and ecosystem structure at larger scales (Freeman et al. 2024).

Using $\delta^{13}C$ as a maize consumption marker, the discrete and long-term, stable hunter gatherer/ farming boundary proposed originally and placed among the Atuel and Diamante rivers (Figures 1 and 3), is not as clear and stable as was expected. Gil et al. (2011) call attention to early characterizations of such a hunter-gatherer/farming boundary, indicating that it should be questioned as a static limit and suggesting that it should be re-interpreted as a shifting zone between geographic expressions of contrasting subsistence modes. The study by Gil et al. (2020c) concludes that the farming frontier in northwestern Patagonia was dynamic in time and space. The authors show how changes in temperature and precipitation over the last 1000 years BP have influenced the use of domestic plants and the hunting of wild big game. Gil et al. (2020c) demonstrated that between 900 and 550 cal. BP, when the climate had warmer and drier summers, there was an associated increase in C_4 resource consumption (e.g., maize). After 550 cal. BP, the climate shifted to wetter and cooler summer conditions—less favorable for C_4 plants. This change coincided with a dietary shift toward wild resources, particularly meat. Importantly, these changes occur during a period in which there is no evidence of significant changes in human population size. It indicates a high resilience to this population that changing its subsistence strategy without a drastic variation in population dynamics.

30 years of Stable Isotope in CWA Archaeology: Then and Now

In this review, we highlight the role of archaeological research with stable isotopes in the study of a complex evolutionary cultural history regarding the incorporation of maize and farming in a shifting late Holocene boundary zone between farming and hunter -gathering. Stable isotope research in CWA began with the measurement of $^{13}C/^{12}C$ in bone collagen and mainly interpreting the proportion of corn in the diet according to the distance to the extremes of the expected values for direct C_3 and C_4 consumption (Fernández and Panarello 1991; Fernández et al. 1999; Novellino and Guichón 1999). Early results were interesting and may have suggested early reliance on maize, but greater depth in isotopic investigations has led to a more subtle early adoption of maize that does not indicate high reliance until after 1000 years BP.

Our review centered on the history of the incorporation of maize as a case of study to show the impact of stable isotope analyses in CWA archaeology. However, the implications of these methodologies are wider than this case study shows. The current agenda has changed from the initial concern about when maize was incorporated into the CWA human diet and how important it was in subsequent processes. Its application has expanded and includes analyses of human (Gil et al. 2011),

animal (Otaola et al. 2018), and plant remains (Aranibar et al. 2023). These studies are helping to define the resource exploitation and management strategies that underscore broader changes in the human diet. The advance in understanding a regional baseline allows us to adjust our archaeological interpretations about mobility and diet. It allows us to know to what degree isotopic variation implies a change in diet and our studies also indicate how environmental, climatic, or other variable influence isotopic variability. The carbon and nitrogen stable isotopes were incorporated to evaluate the proportion of meat and vegetables and even the proportion of different categories of resources in the human diet (Gil et al. 2020b; Mosquera et al. 2022). Recent research builds on these earlier studies with a comprehensive effort to include isotope results in multiproxy analyses framed with questions informed by clear theoretical approaches (Barberena et al. 2020; Freeman et al. 2024; Gil et al. 2020c; Gordón et al. 2018; López et al. 2019; Peralta et al. 2022, 2024). A new perspective emerges with the interaction between stable isotopes on human bones and paleometa-genomics (Lopopolo 2023) as a window to evaluate the relationship between human diet, demography, and health. Sequenced human and microbial DNA, including pathogens, recovered from archaeological samples of farmers and hunter-gatherers permit tackling fundamental questions such as demographic and genetic changes and evaluating their association with the emergence of infectious diseases. The 30-year history of archaeological isotopic research in CWA represents a dynamic history of old and new questions, old and new methodologies, greater statistical sophistication and theoretical depth in its interpretation. Stable isotope research is now a full-fledged research program in CWA and neighboring regions. The application of stable isotopes in CWA has not only improved our knowledge of the human past, but has opened new avenues of research.

Notes

[1]References are limited in order to accept the editorials rules. A more extensive and complementary references list is provided in the Supplementary Materials.

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