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Isotope analysis reveals the relevance of local wild plants in diet and maize as an exotic commodity in pre-Hispanic Patagonia

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The acknowledgment of plants as significant components in hunter-gatherer diets has provided new insights into past interactions between these groups and their environments, as well as between groups with different economies and social structures. The Patagonia region, South American Southern Cone, has been traditionally perceived as dominated by game hunting. This perception has changed by recognizing a broader spectrum of diet, where local wild and exotic domesticated plants, would have been intertwined in these hunter-gatherer lives. However, the nature of the archaeological record preservation obscures an accurate assessment of their role. Here, we quantified the contribution of plants and other resources to hunter-gatherer paleodiets through biomolecular methods and statistical analysis (multivariate and Bayesian). We analyzed the carbon and nitrogen stable isotope composition ($\delta^{13}C_{col}$, $\delta^{15}N$) of human remains ($n = 30$) and fauna/flora ($n = 52$) from Northern Patagonia, and radiocarbon dated specific individuals related to exotic domesticated plants (maize). Our results show that one-third of the overall intake was based on the systematic exploitation of local wild plants, while maize appears to have functioned as an imported edible commodity obtained from distant food producers.

The composition of diets in hunter-gatherer societies has long been a central theme in anthropology and archaeology[1]. Early stereotypes suggested that subsistence in these societies was primarily based on large game hunting[2]. However, this view was challenged decades ago with the recognition that, at least in modern groups, subsistence often relies more on aquatic organisms and plants, with gathering activities proving to be highly productive[3]. Moving beyond the static and typological view of the "(man-)hunter", we now recognize the crucial economic role of women and their contribution to diverse subsistence practices -particularly related to plants[1,4] and embrace the diversity within the continuum of subsistence strategies that combine hunting and gathering[5]. The acknowledgment of plants in hunter-gatherer diets was pivotal not only in challenging the hunting-centric models of early human evolution but also in expanding our understanding of past interactions between these groups and their environments, as well as between groups with different economies and social structures[1].

Hunter-gatherers from Patagonia region (South American Southern Cone) have traditionally been seen as hunters strongly focused on *guanaco* (*Lama guanicoe*)[6,7]. Although the relevance of this prey has been recognized from the Late Pleistocene to post-Hispanic times (e.g.,[8] and references therein), it has become increasingly clear that its relative importance varied across the region, opening an ongoing debate about different intensification strategies -specialization, investment and diversification- (e.g.,[9–11]). The occurrence of a broader spectrum diet in some areas has led to a new perspective on the role of local wild plants in paleodiets and, exotic domesticated ones (maize -*Zea mays*-) as potential exchange goods (e.g.,[12–17]). The poor preservation of plant macroremains and the usual lack of compelling evidence of consumption[18,19], however, have made it difficult to assess accurately the relationship between these hunter-gatherers and plants (local wild and exotic domesticated), ultimately, obscuring their relationship with the environment and with other human groups.

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This work aims to quantify the contribution of plants and other resources to hunter-gatherer paleodiets. We analyzed the carbon and nitrogen stable isotope composition ($\delta^{13}C_{col}$, $\delta^{15}N$) of human remains from Northern Patagonia (Fig. 1) with a special emphasis on individuals with evidence of maize intake that were also radiocarbon dated. Human data was analyzed through a relevant isotope paleoecology and a battery of statistical analyses including Bayesian mixing models to estimate resources proportions (FRUITS[20]).

Results

Human data is listed in Table 1 (see also Supplementary Table S1). Isotope determination was successful in all the analyzed samples. All of them showed acceptable C/N ratios (3.2–3.6) pointing to a good collagen preservation[22]. Collagen $\delta^{13}C$ values ($n = 30$) have medians of -18.6‰ ranging from − 19.7 to − 15‰, and $\delta^{15}N$ of 11‰ ranging from 8.3 to 13.2‰.

The shapes of the data distributions are illustrated through kernel density plots (Fig. 2). Divided by location, the $\delta^{13}C$ kernels show a peak around − 19‰ for NR and a plateau for SF, while $\delta^{15}N$ depicture a smooth bimodal shape around 12‰ and 10‰ for NR, and a distinct peak with a shoulder around 12‰ for SF; without significant differences in any case ($\delta^{13}C$ $W = 59$, $p = 0.517$; $\delta^{15}N$ $W = 34.5$, $p = 0.055$). Distributions of determined sexes greatly overlap for both isotopes; without registering statistically significant differences ($\delta^{13}C$ $W = 82$, $p = 0.5$; $\delta^{15}N$ $W = 96$, $p = 0.138$).

The ecology might be described by the variation of the carbon and nitrogen isotope values among types of resources in visually discrete groups of data (Table 2, see also Fig. 3). First, C_3 plants and fluvial mollusks group together with mean $\delta^{13}C$ values of − 24.9 ± 1.6‰ and − 25.1 ± 1.6‰, and mean $\delta^{15}N$ values of 2.8 ± 3.3‰ and 5.3 ± 0.2‰, respectively. Second, the largest group reunites several terrestrial resources (*guanaco*; large-small herbivores and small omnivores). Fluvial fish is close but separated from the former. The third group is made up marine/coast organisms (fishes, birds; mammals; mollusks). Finally, the C_4 and CAM plants cluster closely.

Descriptive statistics for $\delta^{13}C$ and $\delta^{15}N$ values of reference samples from northeastern Patagonia (see details in Supplementary Table S2).

The integration of the isotope ecology and human collagen values shows that the analyzed individuals are distributed in a space defined by three ^{13}C-depleted resource groups: (1) C_3 plants and fluvial mollusks, (2) fluvial fish, and (3) terrestrial resources (Fig. 3, Supplementary Table S2). According to this, some individuals predominantly consumed C_3 plants (e.g., *Neltuma* sp.*, Geoffroea* sp.) and fluvial mollusks (e.g., *Diplodon* sp.). Having higher values of nitrogen, the rest of the population is scattered in the center of the space and some individuals are closer to a pool of overlapping terrestrial resources (e.g., *guanaco*, Chlamyphoridae, *Ctenomys* sp.*, Rhea* sp.). Individuals showing evidence of maize in their dental calculus are part of the former trend. There is no sample compatible with systematic consumption of either ^{13}C-enriched plants (e.g., C_4 or C_4-behaved) or marine resources. However, one individual (LM_1) shows particularly high $\delta^{13}C$ values that place it in a central position respect to the terrestrial resources.

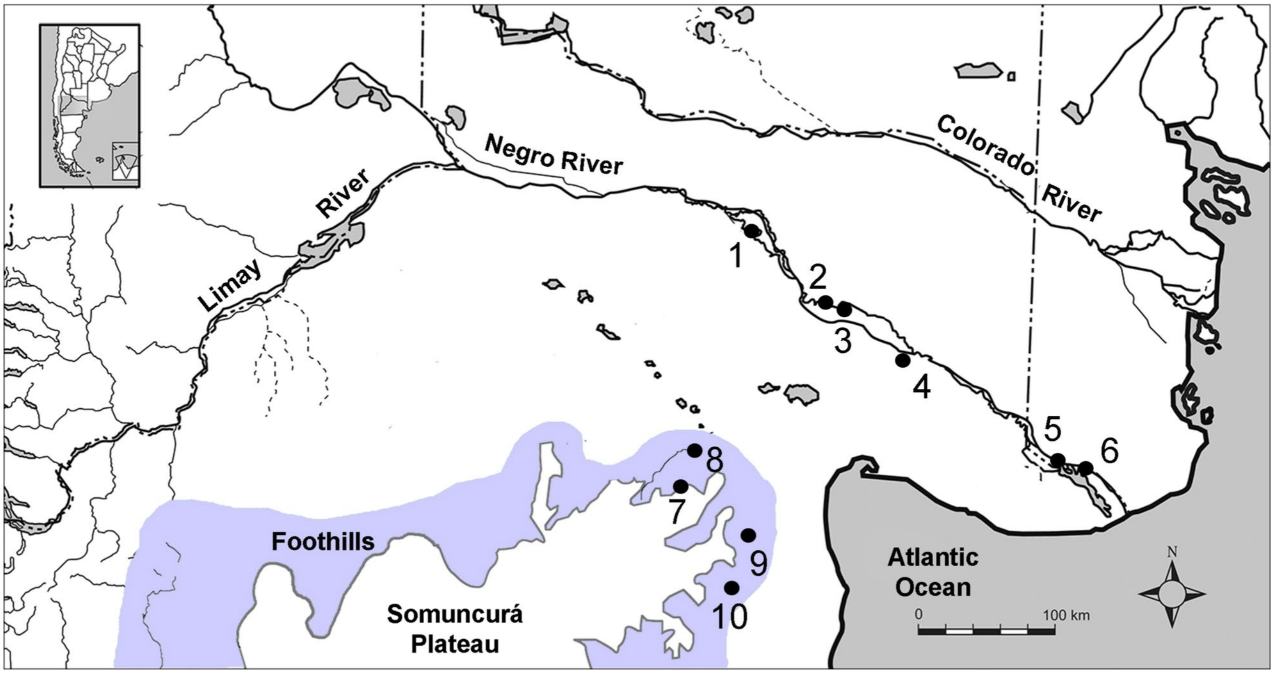

Fig. 1. Archaeological sites with human remains analyzed in this work. Negro River (NR): 1-Fábrega, La Toma, Pomona; 2-Negro Muerto 2 and 3; 3-La Victoria 5; 4-Loma de los Muertos; 5-San Javier; 6-Laguna del Juncal. Somuncurá Foothills (SF): 7-Paja Alta; 8-Valcheta; 9-Cueva Galpón; 10-Arroyo Salado (modified from[21]).

Site	Location[a]	Period[b]	Ind	Sex	Lab-code	$\delta^{13}C_{col}$‰	$\delta^{15}N$‰	$\delta^{13}C_{ap}$‰[c]	C/N	%C	%N	%Coll	F1[d]	F2[e]	Ref[f]
Negro Muerto 3	NR	FLH	NM3_1	M	S_UCT-18661	− 18.9	9.45	− 13.20	3.2	41.9	15.2	3.7	− 6.57	− 0.15	Ref [23]
Negro Muerto 3	NR	FLH	*NM3_2	M	S_UCT-18662	− 18.98	9.98	− 13.12	3.2	40.8	14.9	3.4	− 6.48	0.13	Ref [23]
Negro Muerto 3	NR	FLH	NM3_3	M	S_UCT-18663	− 18.8	10.35	− 12.99	3.2	40.5	14.6	2.0	− 6.23	0.34	Ref [23]
Negro Muerto 3	NR	FLH	*NM3_4	M	S_UCT-18664	− 18.82	9.92	− 13.17	3.2	42.0	15.2	7.0	− 6.4	0.14	Ref [23]
Negro Muerto 3	NR	FLH	NM3_5	F	S_UCT-18665	− 18.63	9.81	− 14.13	3.2	42.4	15.5	3.0	− 6.59	0.48	Ref [23]
Negro Muerto 2	NR	FLH	*NM2_1	F	S_UCT-18681	− 19.28	8.88	− 13.66	3.2	41.9	15.2	2.2	− 7.11	0.38	Ref [24]
Negro Muerto 2	NR	FLH	*NM2_2	F	S_UCT-18682	− 19.07	9.72	− 12.90	3.2	42.2	15.2	2.3	− 6.53	0.13	Ref [24]
Negro Muerto 2	NR	FLH	NM2_3	F	S_UCT-18683	− 19.68	8.93	− 12.92	3.2	42.7	15.5	2.6	− 7.15	− 0.69	Ref [24]
Loma de los Muertos	NR	ILH	LM_1	M	S_UCT-18684	− 14.98	13.24	− 8.34	3.3	41.7	14.9	3.4	− 1.32	0.82	Ref [25]
Loma de los Muertos	NR	ILH	LM_2	F	S_UCT-18685	− 19.33	8.3	− 12.67	3.2	41.7	15.0	3.9	− 6.96	− 1.13	Ref [25]
Loma de los Muertos	NR	ILH	LM_3	F	S_UCT18686	− 18.63	10.3	− 11.49	3.2	42.7	15.4	3.4	− 5.63	− 0.26	Ref [25]
Loma de los Muertos	NR	ILH	LM_4	I	S_UCT18687	− 18.93	8.36	− 12.60	3.3	41.9	14.7	2.7	− 6.63	− 1.07	Ref [25]
La Victoria 5	NR	FLH	LV5_1	F	S_UCT-18697	− 18.25	10.82	− 12.64	3.2	42.9	15.8	5.2	− 5.62	0.57	Ref [26]
La Victoria 5	NR	FLH	LV5_2	F	S_UCT-18698	− 18.2	11.16	− 12.94	3.2	42.8	15.4	3.8	− 5.6	0.9	Ref [26]
San Javier	NR	FLH	SJ_C	F	S_UCT-18711	− 18.06	12.24	− 12.43	3.3	42.7	15.1	5.1	− 5.1	1.39	Ref [27]
San Javier	NR	FLH	SJ_E	F	S_UCT-18712	− 17.71	12.3	− 11.79	3.3	43.2	15.1	3.6	− 4.62	1.23	Ref [27]
San Javier	NR	FLH	SJ_J	M	S_UCT-18713	− 18.31	12.42	− 13.75	3.3	40.3	14.4	5.4	− 5.67	1.99	Ref [27]
San Javier	NR	FLH	SJ_N	M	S_UCT-18714	− 18.06	12.34	− 13.12	3.2	42.7	15.4	6.1	− 5.3	1.73	Ref [27]
San Javier	NR	FLH	SJ_FM	I	S_UCT-18715	− 17.42	12.11	− 12.38	3.3	41.8	14.9	4.6	− 4.64	1.38	Ref [27]
San Javier	NR	FLH	SJ_Ñ	M	S_UCT-18716	− 17.76	13.23	− 12.20	3.2	42.9	15.4	6.3	− 4.59	1.96	Ref [27]
San Javier	NR	FLH	SJ_GQ	I	S_UCT-18717	− 18.44	11.49	-11.95	3.2	43.3	15.6	7.5	-5.38	0.69	Ref [27]
La Toma	NR	FLH	LT_L	F	S_UCT-18718	− 19.02	10.09	− 12.98	3.2	42.7	15.4	5.3	− 6.45	0.14	Ref [28]
Pomona	NR	FLH	Po_1	M	S_UCT-18719	− 18.96	10.2	− 13.47	3.3	43.7	15.7	6.3	− 6.53	0.41	Ref [19]
Fábrega	NR	FLH	Fa_1	F	S_UCT-18720	− 18.98	9.84	− 13.31	3.2	42.7	15.6	5.5	− 6.57	0.13	Ref [27]
Paja Alta	SF	FLH	PA_1	M	S_UCT-18760	− 19.61	11.18	− 14.65	3.4	44.4	15.3	4.6	− 7.17	1.4	Ref [27]
Arroyo Salado	SF	FLH	ArrS_1	I	S_UCT-18761	− 17.39	11.58	− 11.12	3.3	41.2	14.4	5.7	− 4.34	0.55	Ref [27]
Valcheta	SF	FLH	Val_1	F	S_UCT-18762	− 19.49	12.11	− 14.26	3.2	43.7	15.8	6.5	− 6.75	1.84	Ref [27]
Cueva Galpón	SF	ILH	CvG_1	I	S_UCT-18749	− 15.96	12.14	− 10.91	3.2	42.2	15.4	1.5	− 3.11	1.01	Ref [29]
Cueva Galpón	SF	ILH	CvG_2	F	S_UCT-18750	− 16.71	12.25	− 11.26	3.6	44.8	14.7	7.3	− 3.74	1.12	Ref [29]
Cueva Galpón	SF	ILH	CvG_3	I	S_UCT-18751	− 18.47	12.59	–	3.4	44.1	14.9	6.4	–	–	Ref [29]

Table 1. Contextual and isotope data ($\delta^{13}C$, $\delta^{15}N$) of human remains analyzed in this work. [a]NR: Negro River; SF: Somuncurá Foothills [b]ILH: Initial Late Holocene (~ 3000–1500 years BP); FLH: Final Late Holocene (~ 1500–300 years BP). Assigned based on radiocarbon dates and/or contextual information [c]values taken from Serna et al.[30] [d/e]values of the axes calculated by the paleodiet discriminant analysis (see Fig. 4) [f]correspond to chronology and contextual information. *Individuals with microfossil evidence of maize in dental calculus (see details in Saghessi et al.[16]).

Figure 4 shows the integration of the human isotope values ($\delta^{13}C_{col}$, $\delta^{13}C_{ap}$, $\delta^{15}N$) into the discriminant space of the multivariate diet reconstruction model[31]. According to this classification, the overall population exhibits a predominance of C_3 diets that ranges from 100% to ≥ 65% C_3 protein (cluster 1 and 4, respectively). Individuals with microfossil evidence of maize intake are part of these clusters. Deviated from this trend, one individual (LM_1) moves along the F1 axis towards the discriminant space of C_4 diets (30:70 C_3:C_4, > 50% C_4 protein; cluster 2).

Based on the distribution of the human samples in relation to the ecology (Fig. 3), three statistically different food groups (pairwise PERMANOVA $p < 0.05$, Supplementary Table S3 were defined to evaluate their contribution to the paleodiets (Table 3).

Figure 5 shows the mean contributions of the foodgroups for the entire sample pooled together (see individual results in Supplementary Table S4). The estimate shows that human diet in the study area have a mean intake of 52% of terrestrial resources (oscillating between ~ 40–60% at percentile 25–75th), 30% of C_3 plants (~ 10–40% at percentile 25–75th), and 18% of fluvial fish (~ 10–20% at percentile 25-75th) (Fig. 5a). Those individuals with maize in their dental calculus show less terrestrial consumption (29–44%) with an increase of plants (40–58%) (Fig. 5b–e). According to this model, there is one outlier (LM_1) whose terrestrial intake is ~ 90% (Fig. 5a, f). The position of LM_1 within the space of the isotope ecology (Fig. 3) and the results from the discriminant analysis (Fig. 4) foster the creation of an alternative model for this outlier using the following endmembers: C_3 plants, Terrestrial animals and C_4/CAM plants ($n = 2$, $\delta^{13}C_{protein} = -\ 13.2 \pm 0.2$, $\delta^{15}N_{protein} = 0.8 \pm 0.2$, $\delta^{13}C_{energy} = -\ 10.7 \pm 0.2$). This alternative model decreases to 72% the amount of terrestrial intake, increases to 6% C_3 plants and poses a 22% for C_4/CAM (Fig. 5g).

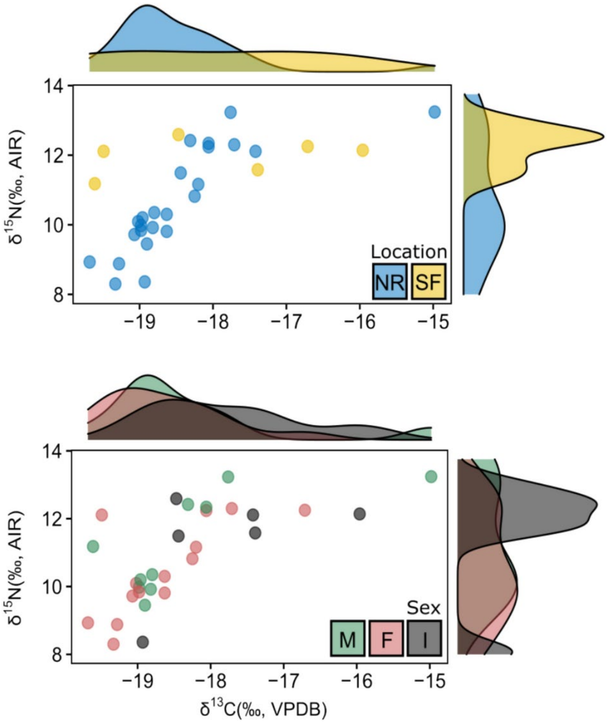

Fig. 2. Kernel density estimation of δ^{13}C and δ^{15}N by Location (NR -Negro River-, SF -Somuncurá Foothills-) and Sex (M -Male-, F -Female-, I -Indet.-).

		δ^{13}C ‰			δ^{15}N ‰		
Resource Type	**n**	**Mean ± SD**	**Min**	**Max**	**Mean ± SD**	**Min**	**Max**
C3 plant	19	− 24.9 ± 1.6	− 28.4	− 22.4	2.8 ± 3.3	− 2.5	9.1
Fluvial mollusk	2	− 25.1 ± 1.6	− 26.2	− 24.0	5.3 ± 0.2	5.1	5.4
Guanaco	27	− 19.5 ± 1.1	− 21.4	− 17.6	6.6 ± 1.7	4.3	10.5
Terrestrial large herbivore	19	− 20.4 ± 1.9	− 24.9	− 17.2	7.6 ± 1.9	4.4	11.4
Terrestrial small herbivore	11	− 20.1 ± 1.7	− 22.3	− 16.9	6.1 ± 1.3	3.7	8.0
Terrestrial small omnivore	6	− 18.5 ± 3.5	− 24.7	− 14.9	8.8 ± 2.5	5.0	11.0
Fluvial fish	6	− 23.2 ± 3.4	− 29.5	− 19.4	9.2 ± 1.3	7.9	11.2
Marine fish	6	− 13.8 ± 1.0	− 15.5	− 12.5	17.9 ± 1.4	16.7	20.4
marine bird	7	− 14.7 ± 1.4	− 16.7	− 12.2	18.3 ± 1.7	16.8	21.1
Marine mammal	4	− 13.0 ± 2.3	− 15.8	− 11.1	22.2 ± 1.0	20.7	22.8
Marine mollusk	8	− 13.0 ± 1.1	-14.9	− 12.0	14.5 ± 1.7	12.3	16.9
C4 plant	1	− 11.0	–	–	0.9	–	–
CAM plant	1	− 11.3	–	–	0.6	–	–

Table 2. Isotope ecology.

Discussion

Population diet

The overall population diet follows a ^{13}C-depleted pattern that includes terrestrial, fluvial and vegetal components; without showing any sex or location driven difference among individuals (Fig. 2). The integration of the analyzed human and ecological isotope data points to the exploitation of a wide diversity of ^{13}C-depleted resources or a C_3 diet in terms of the base of the trophic chain (Fig. 3). These diets are featured by a range of terrestrial resources that include large to small fauna (e.g., artiodactyls, birds, armadillos, rodents), fluvial resources (fish and mollusks), and plants with C_3 photosynthetic pathways. The dietary characterization provided by the discriminant analysis also supports the predominance of C_3 resources (Fig. 4). Almost all the individuals fall either in cluster 1 (100% C_3 diets) or the overlapping region between the former and cluster 4 (70:30 C_3:C_4 diet, ≥ 65% C_3 protein). Given that Northern Patagonia is dominated by plants following the C_3 photosynthetic pathway (e.g., [33–35]), the most parsimonious interpretation is that individuals in the overlapped area were most likely consumers of full C_3 diets. The estimates of the resource contribution to diet for the population indicate a predominance of terrestrial animals

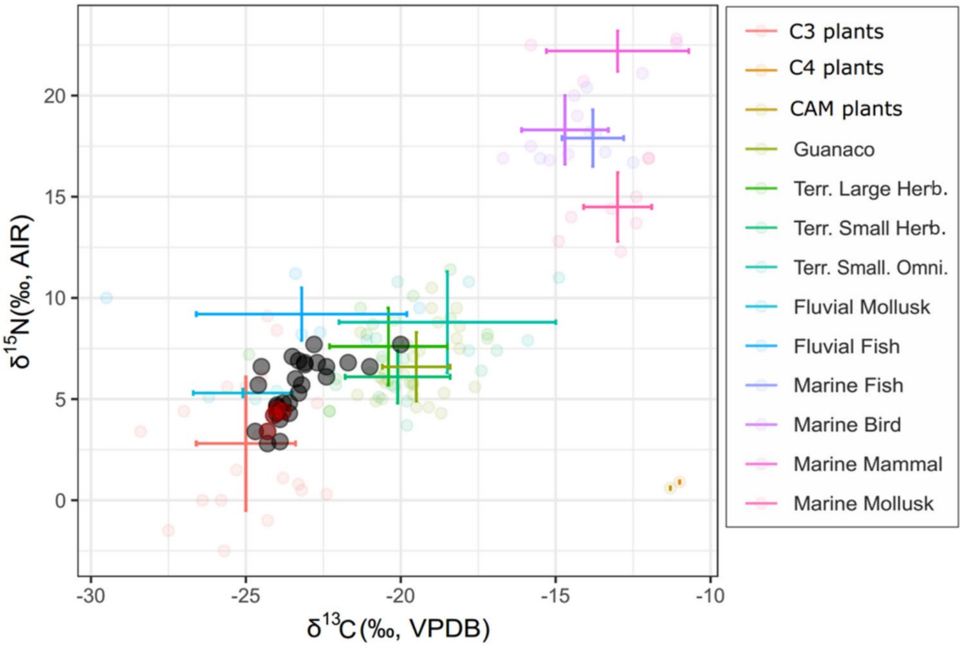

Fig. 3. Isotope ecology and human δ^{13}C and δ^{15}N values for the study area (Tables 1 and 2). Whiskers correspond to 1 SD from the mean. Gray dots represent human samples, in red individuals with microfossil evidence of maize in dental calculus.

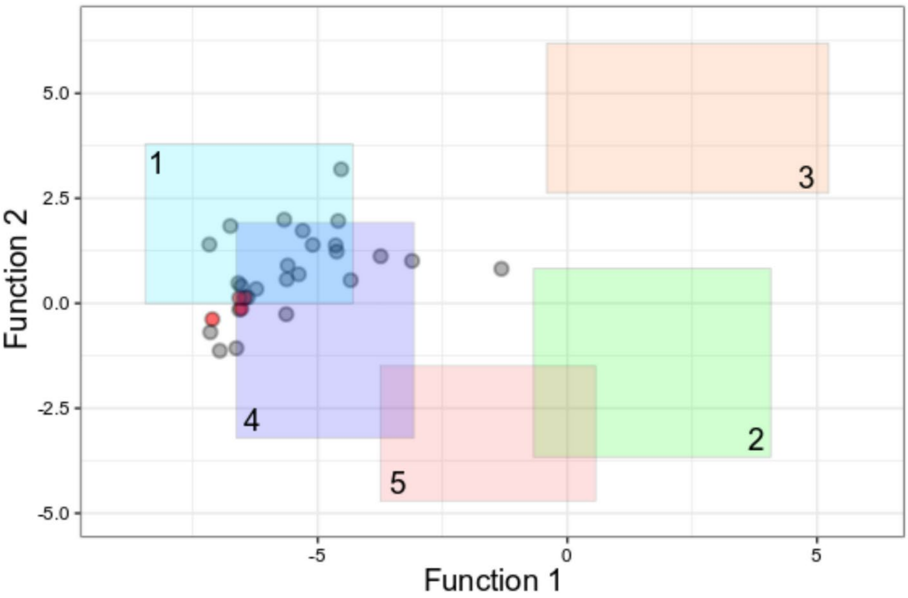

Fig. 4. Discriminant function analysis for paleodiet reconstruction based on $\delta^{13}C_{col}$, $\delta^{13}C_{ap}$, δ^{15}N isotope values[31]. Diet clusters are: (1) 100% C_3 diet/protein; (2) 30:70 C_3:C_4 diet, > 50% C_4 protein; (3) 50:50 C_3:C_4 diet, marine protein; (4) 70:30 C_3:C_4 diet, 65% C_3 protein; (5) 30:70 C_3:C_4 diet, 65% C_3 protein. Red dots correspond to individuals with microfossil evidence of maize in dental calculus.

(52%) followed by C_3 plants (30%) and fluvial fish (18%) (Fig. 5a). Global trends from the ethnographic record support these contributions. The data analysis of more than 200 hunter-gatherer societies worldwide shows that when the ecological conditions allow it, most of them (73%) derive > 50% of their subsistence from animal foods, whereas a small percentage derives > 50% of diet from plants[36].

These results are aligned with the overall broad spectrum of diet inferred by the archaeological record from the study area. Terrestrial animals dominate this contribution, representing around the half of the dietary intake of the individuals. Given the fairly permanent extensive offer of resources available in the area; this contribution

Resource group	n	$\delta^{13}C_{protein}$‰	$\delta^{15}N_{protein}$‰	$\delta^{13}C_{energy}$‰
Terrestrial animals	63	− 21.8 ± 1.8	7 ± 1.9	− 27.8 ± 1.8
Fish[a]	28	− 23.7 ± 0.6	8.5 ± 0.4	− 29.7 ± 0.6
C3 plants[b]	19	− 26.9 ± 1.6	2.6 ± 3.3	− 24.4 ± 1.6

Table 3. Endmembers/Resource groups for Bayesian paleodietary reconstruction. Mean and SD of the estimated protein and energy $\delta^{13}C$ and $\delta^{15}N$ values for resource groups. This data represents bone collagen and bulk plant values [a]This category is expanded using the compilation of Fernandes et al.[32] [b]Freshwater mollusks were excluded given the lack of adequate conversions (see[32]).

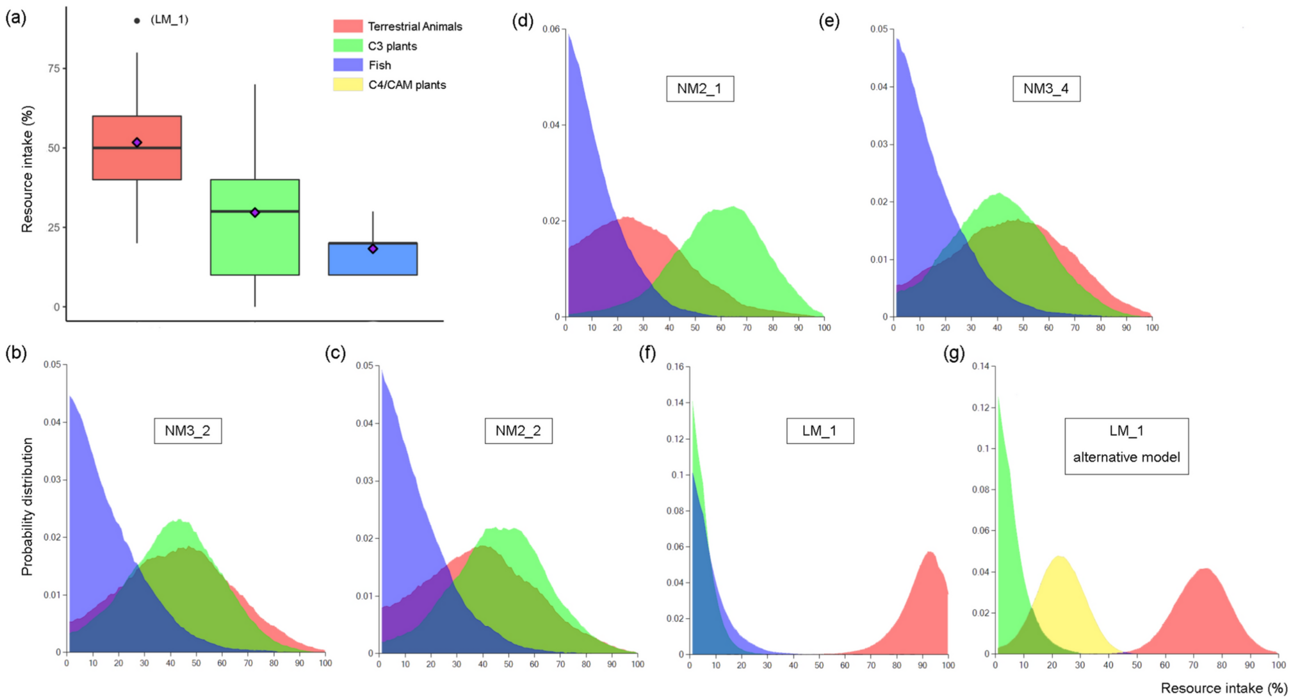

Fig. 5. Resource contribution to diet through mixing models. (a) Boxplot of the mean estimates of resource contribution to diet for the entire sample (Supplementary Table S4). Purple diamonds represent the total means. (b–g) Estimates of resource contribution for those individuals with microfossil evidence of maize in dental calculus and potential C_4-like diet.

is within the expectations. Previous archaeological models pointed to a diverse exploitation of species from different sizes and niches in the Negro valley[9,19,23], with a decreasing diversity southward in the Somuncurá Foothills[37]. It is worth pointing that our category of terrestrial animals encompasses a wide repertory of small (e.g., Clamyphoridae) to large preys (e.g., *guanaco*) (Supplementary Table S2). Although the isotope data does not allow us to point to specific animals within this group, the results align with all the evidence that pose the *guanaco* as a central piece of the Patagonian economy through the entire Holocene ([9,13,38]and references therein;[19,39,40]). However, local dietary choices (see[40]), and the intrinsic elevated biodiversity found in the valleys from this region and nearby, moderate the relative importance of this prey (e.g., Negro River[9]; Chubut River[10]; Colorado River[11]; Limay River[41]). It seems more realistic a scenario of alternation and complementation between the *guanaco* and smaller preys. This equilibrated exploitation has been noted in northwestern Patagonia mostly based on the zooarchaeological record ([42] and references therein). Although increasing the organism mass usually bears more elevated percentages of body fat[36], the *guanaco* meat is considerably lean; lacking of fat that small prey provides, at the time that the latter are always available and in high densities[40,43]. While some degree of *guanaco* specialization might have existed (e.g., hunting blinds for communal hunting[44,45]; cf.[46]), the alternation and complementation between animals with different body compositions is most likely the type of exploitation carried out at least in Northern Patagonia.

According to the mixing models, plants accounted for approximately one-third of the dietary intake of the population. We note that this contribution could be inflated to some degree by fluvial mollusks, given their similarities with C_3 plants in carbon isotope values (Table 2). However, the zooarchaeological record suggests their circumstantial and variable exploitation[9,19]. A dietary shift towards increased plant consumption, occurring approximately 1500 to 1000 years BP, has been proposed for some areas based on various lines of evidence: (1) increasing frequency of grinding tools, pottery, and plant macroremains in the archeological record (e.g.,[12,47–50]);

(2) higher prevalence of dental diseases (e.g., caries)[49,51–53], and (3) increased isotopic signal of plant intake in collagen and bioapatite of human remains[33,35]. Our results show that plants made an important contribution to the diet, but do not seem to reflect a shift towards increased consumption of plants during the Late Holocene. The plant contribution for the Negro valley corresponding to the Initial Late Holocene (ILH, Table 1) is 43% in average (Supplementary Table S4). An assessment of the oral health of the same sample also shown that there is no evidence of diet change -particularly rated to plant intake- through the Late Holocene[25,54], which strongly agrees with paleopathological reports from neighboring regions[12,55]. This preliminary observation suggests that a radical process such as a dietary *shifts* might have not occurred, at least in the same way everywhere, and that the important role of plants could have pre-dated technological innovations (e.g., pottery) also occurring at that time; both hypothesis worth developing in the future.

C_4-like diet: domesticated and local

The diet of one individual from the Negro valley -LM1- is remarkably close to C_4 diets (Fig. 4). The consideration of its central position in the bivariate isotope space (Fig. 3) added to its extreme terrestrial contribution of ~ 90% (Fig. 5a, f), fostered the creation of an alternative model for this outlier replacing fluvial fish for C_4/CAM as endmember. This model is more balanced (Fig. 5g), and quantifies the C_4-like isotope signal detected by the discriminant analysis. Given the availability of C_3/C_4 resources in the area, the contribution calculated by the mixing model (22%) might be more accurate than the discriminant. The C_4-like diet of this individual might be discussed in terms of the exploitation of maize, CAM, and wild C_4 plants. Although the recent findings of maize in dental calculus from the area[16] pose the intake of this domesticated C_4 as a potential explanation, some factors make it unlikely. The radiocarbon date of this individual (~ 2000 years BP) is outstandingly early in relation to maize occurrence in the area or even in the closest potential production centers (see discussion below). In turn, its dental calculus shows microfossil evidence of exclusively local plants. The identification of starch grains of *Tropaeolum/Alstromeria* would point to a catchment area in the Patagonian Forest or the Atlantic coast[16], being the latter the most likely location of provenance for this individual given its strontium isotope values[21,56].

CAM plants are also an alternative available in this region that mimics C_4 plant isotope values (*Cereus aethiops*[31], *Opuntia* sp.[57,58]), and carbon enriched tissues are usually attributed to them (e.g.,[35,57,59]). However, aside from exceptions[60], there is no direct evidence of its exploitation (macro/microremains). Most of the organic residue analysis carried out on pottery in the region has detected plants (e.g.,[61–63]), but never reported potential CAM biomarkers (e.g., long-chained alcohols for *Opuntia* sp.[64]). Alternatively, there are wild C_4 plants that might have contributed (*Portulaca* sp.[58]; *Panicum* sp. –Supplementary Table S2). The morphology of the starch grains found in the dental calculus of LM_1 suggests that *Panicum* sp. -Poacea- was consumed[16,17]. The highly cariogenic dental profile of this particular individual[25] might be explained, in turn, by the elevated richness of *Panicum* sp. in carbohydrates -major macronutrient- (e.g., ranging from 65.1 to 75.3 g/100 g total carbohydrates[65]). As mentioned, these wild C_4 plants has never been considered in the Patagonian paleodiet discussion, even when there is abundant ethnohistorical records of its consumption[13,14], and archaeological evidence of their exploitation in neighboring regions[66] and in other parts of world (e.g.,[67]). This case highlights the need to consider empirically supported alternative options for our paleodiet inferences, and opens up a new discussion on the role of wild grasses in hunter-gatherer diets.

Maize dispersion and implications

The four individuals with maize in their dental calculus follow the population pattern of C_3-dominant diet (Figs. 1–2), with a decrease in terrestrial consumption (29–44%) and an increase of plants (40–58%) (Fig. 5b–e). Both isotope composition of biomineralized tissues and dental calculus represent widely different time spans, where the former represents an average of several years and the latter months. This incongruence between long- and short-time spans implies that maize was consumed only by some individuals and not systematically. The lack of biomolecular[21,56] and contextual evidence[23,24,68] pointing these individuals as migrants from production areas, suggests that maize entered this region. Although there are also records of microremains of maize in pottery and grindstones from North Patagonia and neighboring regions[49,69,70], the way that this crop entered the area to be consumed locally is still unknown. Apart from maize, there are records of other exotic edible plants circulating in the area: *mistol* (*Ziziphus mistol*)[71], *Lagenaria siceraria*[72], *Cucurbita maxima*[73]; underground storage organs (*Tropaeolum/Alstromeria*) and *pehuén* (*Araucaria araucana*)[17]. The regional exchange of food is a well-known strategy to mitigate risks associated with environmental uncertainty (e.g.,[74]), but this is unlikely at least in our case given the undetectable contribution of maize to the overall diet and the low frequency of individuals bearing microfossil evidence. Instead, rather than a significant diet component, it seems that maize operated as an exotic edible commodity.

The new radiocarbon dates of maize consumers presented here (NM2_1 = 1660 ± 60 [1696–1370 cal BP], NM3_2 = 850 ± 50 [1535–1316 cal BP], NM3_4 = 840 ± 40 [788–665 cal BP] (Fig. 6, Supplementary Table S5) change our current knowledge on the maize dispersion and potential implications. One of them (NM2_1: 1660 ± 60 BP) represents the earliest direct evidence of maize consumption far away from potential productions areas in Patagonia (Supplementary Fig. S1). Other early evidence is indirect (Michacheo[49]) and/or located around the border of the production areas (Gruta del Indio and Cueva Ponontrehue[75]). The long distance -hundreds km- that separates this individual from these areas, either Southern Chile to the west or central western Argentina to the northwest, makes the date striking and intriguing. Ruling out the hypothesis of local production, it remains clear either that maize is likely earlier and/or it was spread -as an exotic edible commodity- faster than we thought, reaching farther marginal areas such as the Negro valley in a short period. The earliest records from Southern Chile and central western Argentina are ~ 1200–1100 BP[76] and ~ 2000–1500 BP[77], respectively; so, maize probably came from the latter. Moreover, the dates point to two chronologically distant events of consumption, one around 1700–1300 cal BP, and the other one ~ 790–650 cal BP (Fig. 6, Supplementary Table

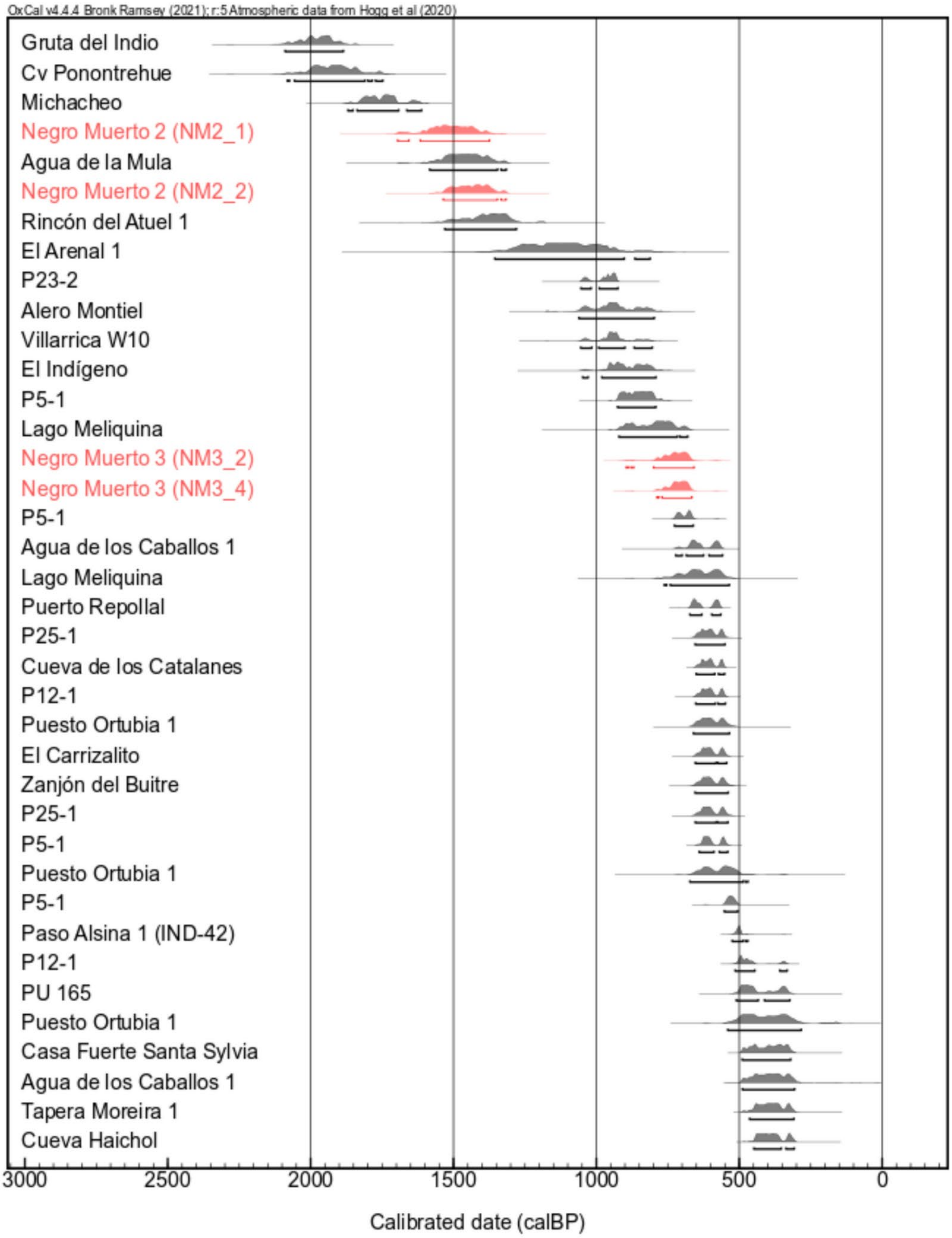

Fig. 6. Calibrated radiocarbon dates (cal BP) of maize evidence within the periphery and its proximity of the southernmost production area (see Supplementary Fig. S1 and Table S5).

S5). In contrast to the later nearby chronological evidence that is more continue (Paso Alsina 1 = 525–470 cal BP and Tapera Moreira 1 = 464–309 cal BP), it seems that at least in earlier moments maize could have traveled in pulses; hypothesis that requires more data to be better drawn and contrasted.

Conclusion

We demonstrated that the broad spectrum of diet inferred from the archaeological record aligns with human dietary patterns, with plants contributing approximately one-third of the overall intake. This contribution came from the systematic exploitation of local wild C_3 and, potentially, some C_4 plants, while maize did not play a significant role. Rather, this exotic domesticated crop appears to have functioned as an imported edible commodity, entering the region in sporadic pulses, as suggested by the new radiocarbon dates. While we have quantified the important role of plants in these hunter-gatherer diets, we also provide a new platform for testing and discussing the interactions between these groups and geographically distant food producers.

Materials and methods

Samples and laboratory processing

The samples come from sites located on the Negro River valley (NR) and Somuncurá Foothills (SF) dated to the Late Holocene (ca. 3000–250 years BP) (Fig. 1, Table 1). The sites from the valley are located in sandy elevations and usually contain remains of residential occupations (e.g., lithic artefacts, pottery sherds, faunal bones) associated with human burials, while the others are located in natural rock shelters and rock mound burials[68,78,79].

We extracted the bone collagen of 30 samples of human adults of different sex coming from the Negro River and Somuncurá Foothill areas (Fig. 1, Table 1, Supplementary Table S1). Sampling focused on ribs for offering long-term average in terms of isotopic composition (> 10 year[80]). We generated a local isotope ecology ($n = 52$) by sampling a diversity of archaeologically relevant species of plants and vertebrates to expand and integrate into the available repertoire of resource isotope data (Table 2, Supplementary Table S2). These samples come from template to cold arid/semi-arid environments that are unlikely to alter the original composition of the collagen[35,58,81], and none of them showed any signs of pathological conditions that might also impact this study.

Laboratory procedures for the isotope analysis ($\delta^{13}C$, $\delta^{15}N$) were carried out at the Stable Light Isotope Laboratory at the University of Cape Town (South Africa). Plant samples were washed thoroughly with MilliQ water, dried (60 °C for at least 48hs), reduced to small pieces and weight out into tin capsules ready for isotope analysis (~ 0.5 mg for carbon, and ~ 3 mg for nitrogen). Bone samples were mechanically cleaned by removing the outer layer by abrasion using a Dremel drill. Collagen extractions were done following the Longin[82] method with additional ultrafiltration[83]. Samples (~ 300 mg) were demineralized in 0.5 M HCl solution at 5 °C, rinsed three times with deionized water until pH became neutral, and gelatinized at 70 °C for 48 h using a heater block. Solutions were filtered with a 9 ml EZEE-filters (Elkay, UK) to remove small (< 8 µm) particles and ultrafiltered with 30 kDa ultrafilters (Amicon, Germany) using a centrifuge at 2500 rpm. The final solution was then frozen and lyophilized for 48 h. About 0.5 mg of collagen per sample -in duplicates- was weighed into tin capsules, and loaded into the mass spectrometer.

The carbon and nitrogen isotope ratio measurements were carried out using a Finnigan Delta plus XP continuous-flow IRMS (Thermo Fisher Scientific, USA) after being combusted in an elemental analyser Flash EA 1112 interfaced with it (Thermo Fisher Scientific, USA). Stable isotope ratios were expressed relative to the V-PDB (Vienna PeeDee Belemnite) for carbon, and stable nitrogen isotope ratios were measured relative to the AIR (atmospheric N2) for nitrogen, using the δ notation in parts per thousand (‰). Standards used were in-house standards MG (expected: $\delta^{13}C = -20.05‰$, $\delta^{15}N = 7.5‰$; analyzed together with samples from this study [n = 58]: $\delta^{13}C - 20.57 \pm 0.19$ ‰ , $\delta^{15}N + 7.49 \pm 0.09$ ‰), seal (expected : $\delta^{13}C = -11.97‰$, $\delta^{15}N = 15.84‰$; analyzed together with samples from this study [n = 54] : $\delta^{13}C - 12.02 \pm 0.20$ ‰ , $\delta^{15}N + 15.78 \pm 0.10$ ‰), and valine (expected : $\delta^{13}C = -26.80‰$, $\delta^{15}N = 12.14‰$; analyzed together with samples from this study: $\delta^{13}C = -26.78 \pm 0.19$ ‰ , $\delta^{15}N = 12.21 \pm 0.09$ ‰). Each standard had been calibrated against international standard materials NBS 21, IAEA N1 and N2 and standards exchanged with other laboratories. The reproducibility of repeated measurements of standard materials was ≤ 0.2‰ for $\delta^{13}C$ and ≤ 0.1‰ for $\delta^{15}N$. Two blank capsules were analyzed per tray run, all of which presented no peak during analysis.

Statistical procedures and data adjustments

The non-parametric Wilcoxon rank sum test, suitable for small unequal sized and independent samples[84], was used to test whether the median distributions of the isotopes values ($\delta^{13}C_{col}$, $\delta^{15}N$) differ by sex and location at a significance level of 0.05 (R 4.1.1 package *rstatix*). Isotope data underwent different adjustments to make it suitable for analysis. Modern resources that are part of the isotopic ecology were Suess Effect-corrected by + 1.5‰[85]. To infer palaeodiet we applied qualitative, quantitative and Bayesian approaches that complement each other. We first plotted in the same bivariate isotope space the ecology and human samples, where the latter collagen values were adjusted to diet with + 5‰ for $\delta^{13}C$ and + 5.5‰ for $\delta^{15}N$**[20]**. The quantitate approach incorporates all the isotope data ($\delta^{13}C_{ap}$, $\delta^{13}C_{col}$, and $\delta^{15}N$) into a previously built multivariate discriminant model that distinguishes five dietary clusters[31]. Finally, we used Bayesian mixing model Food Reconstruction Using Isotopic Transferred Signals (FRUITS) to quantify the contribution of different resources into individual diets[86]. A pairwise PERMANOVA was used to test whether the food groups (i.e., end members of the model) differ in their isotope ratios (R 4.1.1 package *vegan*). Although some plants might be relatively high in protein (e.g., Fernandes et al.[86]: Table 3), they are typically characterized by a much higher energetic content (carbohydrates) than protein. The $\delta^{13}C$ value of collagen is composed by fixed proportions of macronutrients -proteins, lipids, carbohydrates- with approximately three quarters contribution from protein[32]. FRUITS is capable of handling different macronutrients contributing in varying proportions towards the consumer isotopic signal. Values ($\delta^{13}C$, $\delta^{15}N$) from the food groups were disaggregated into macronutrients following broadly used offsets: $\Delta^{13}C_{protein-collagen} = 2‰$, $\Delta^{13}C_{lipids-collagen} = 8‰$, and $\Delta^{15}N_{protein-collagen} = 0‰$ for animals; $\Delta^{13}C_{protein-collagen} = 1‰$, $\Delta^{13}C_{lipids-collagen} = 7‰$, and $\Delta^{15}N_{protein-collagen} = 0‰$ for fish; and $\Delta^{13}C_{bulk-protein} = 2‰$ and $\Delta^{13}C_{bulk-carbohydrates} = +0.5‰$ for plants[20,59,87]. Our FRUITS model is unrestricted (i.e., no priors), concentration independent, and accounts for dietary routing in $\delta^{13}C_{col}$ and $\delta^{13}C_{ap}$ values with the following equations under an uncertainty of 0.5 ‰: $\delta^{13}C_{col} = 4.8 + 0.74 * \delta^{13}C_{protein} + 0.26 * \delta^{13}C_{energy}$; (2) $\delta^{13}C_{ap} = 10.1 * \delta^{13}C_{diet}$ (see[32]). Individuals with evidence of maize intake (Table 1) were radiocarbon dated at the Laboratorio de Radiocarbono (LATYR, CIG-CONICET) at the Universidad Nacional de la Plata. Dates were calibrated using OxCal 4.4 with the SHCal20 curve[88,89].

Data availability

Data Availability: All relevant data are within the article and its Supplementary files.

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Conceptualization: AS, LP. Data curation: AS, LGV, DS. Formal analysis: AS, LGV. Funding acquisition: DCSG, LP. Investigation: AS, LP, LGV, DS, EM, VR, JMC, DCSG. Methodology: AS, DCSG, LGV. Writing—original draft: AS, LP. Writing—review & editing: AS, LP, LGV, DS, EM, VR, JMC, DCSG.

Competing interests

The authors declare no competing interests.

Additional information

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