



# A comparison of nitrogen isotope compositions of charred and desiccated botanical remains from northern Peru

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## Abstract

Isotopic measurements of plant remains from archaeological sites are now routinely used to reconstruct agricultural practices in the Old World. These studies use charred botanical remains as the analytical substrate because (1) these are the materials that are commonly preserved in the archaeological record and (2) the integrity of the isotopic compositions of archaeological plant remains that are uncharred or desiccated has been questioned, particularly for  $\delta^{15}\text{N}$ . By comparing charred and uncharred plant remains from two Late Moche (AD 600–800) sites in the Jequetepeque valley on the north coast of Peru—San José de Moro and Cerro Chépén—we sought to directly test the assumption that the isotopic compositions of desiccated plant remains are not representative of the isotopic compositions of the living plants. The average  $\delta^{15}\text{N}$  values of charred ( $+11.3 \pm 4.4\text{‰}$ ) and desiccated ( $+10.1 \pm 5.1\text{‰}$ ) remains from these two sites did not significantly differ from one another, suggesting that uncharred plant remains can produce reliable isotopic measurements under some circumstances. Furthermore, the relatively high  $\delta^{15}\text{N}$  values of the plant remains from these two sites are consistent with the ancient use of composted camelid manure as a fertilizer.

**Keywords** Archaeobotany · Stable isotopes · Andes · Taphonomy · Charred remains · Desiccated remains

## Introduction

Bulk carbon and nitrogen isotopic measurements of macrobotanical remains from archaeological sites have been employed with increasing regularity over the last decade to detect the use of manures, assess water use efficiency, identify crop provenance, and establish isotopic baselines for plants that would have been consumed by ancient human populations (Aguilera et al. 2008; Fiorentino et al. 2012; Bogaard et al. 2013, 2018; Araus et al. 2014; Vaiglova et al. 2014a; Styring et al. 2017; Alagich et al. 2018). Although

this recent surge in scholarship has focused on materials from Old World archaeological sites, the first research investigating the feasibility of isotopic measurements of archaeobotanical remains by DeNiro and Hastorf (1985) examined materials from the central Andean region of South America. One of the key findings of this pioneering work was that charred plants produced stable carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) isotopic compositions comparable to those of modern plants, whereas desiccated plants produced  $\delta^{15}\text{N}$  values that were often unusually high, frequently between +10 and +20‰ and as high as +46‰; a specific mechanism causing these patterns could not be identified, but some type of post-burial alteration of the organic matter was suspected.

Based on these observations, there has frequently been an implicit or explicit assumption that only charred archaeobotanical materials are suitable for isotopic analyses. There are, however, two major issues with this logic. All of the non-charred (desiccated) samples included in the DeNiro and Hastorf study were collected from low altitude sites on the arid coast of Peru, while all of the charred samples were collected from high altitude sites located inland. Plants growing in the coastal deserts of Peru may be characterized by significantly higher  $\delta^{15}\text{N}$

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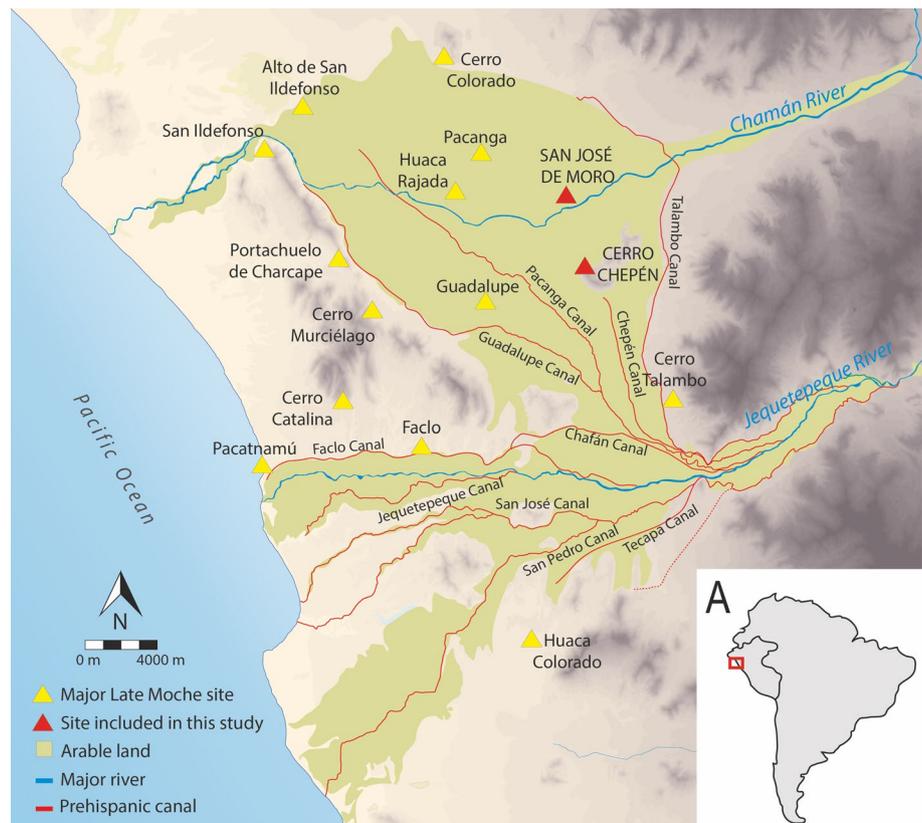
than those growing at higher altitudes due to the effects of water availability on the terrestrial N-cycle (Austin and Vitousek 1998; Handley et al. 1999); such a pattern has been observed in modern wild plants in the Moche valley of northern Peru (Szpak et al. 2013). Second, plants that have been fertilized with seabird guano, which was abundant off the coasts of Peru and Chile (Cushman 2013), are characterized by unusually high  $\delta^{15}\text{N}$  values (+20 to +45‰) (Szpak et al. 2012a, b, 2014a). Therefore, because of both environmental and cultural factors, it is possible that the unusually high  $\delta^{15}\text{N}$  values observed by DeNiro and Hastorf (1985) were not the product of some alteration in the burial environment, but rather reflections of the original nitrogen isotope compositions of these plants. This study was designed with two objectives in mind. First, we sought to further investigate the patterns observed by DeNiro and Hastorf (1985) through the analysis of both charred and desiccated plant remains from archaeological sites in the Jequetepeque valley of northern Peru. By using two classes of remains from a single environmental context, we aimed to replicate their results. Second, this research functioned as a pilot study to assess the pre-hispanic use of fertilizers in this valley.

## Materials and methods

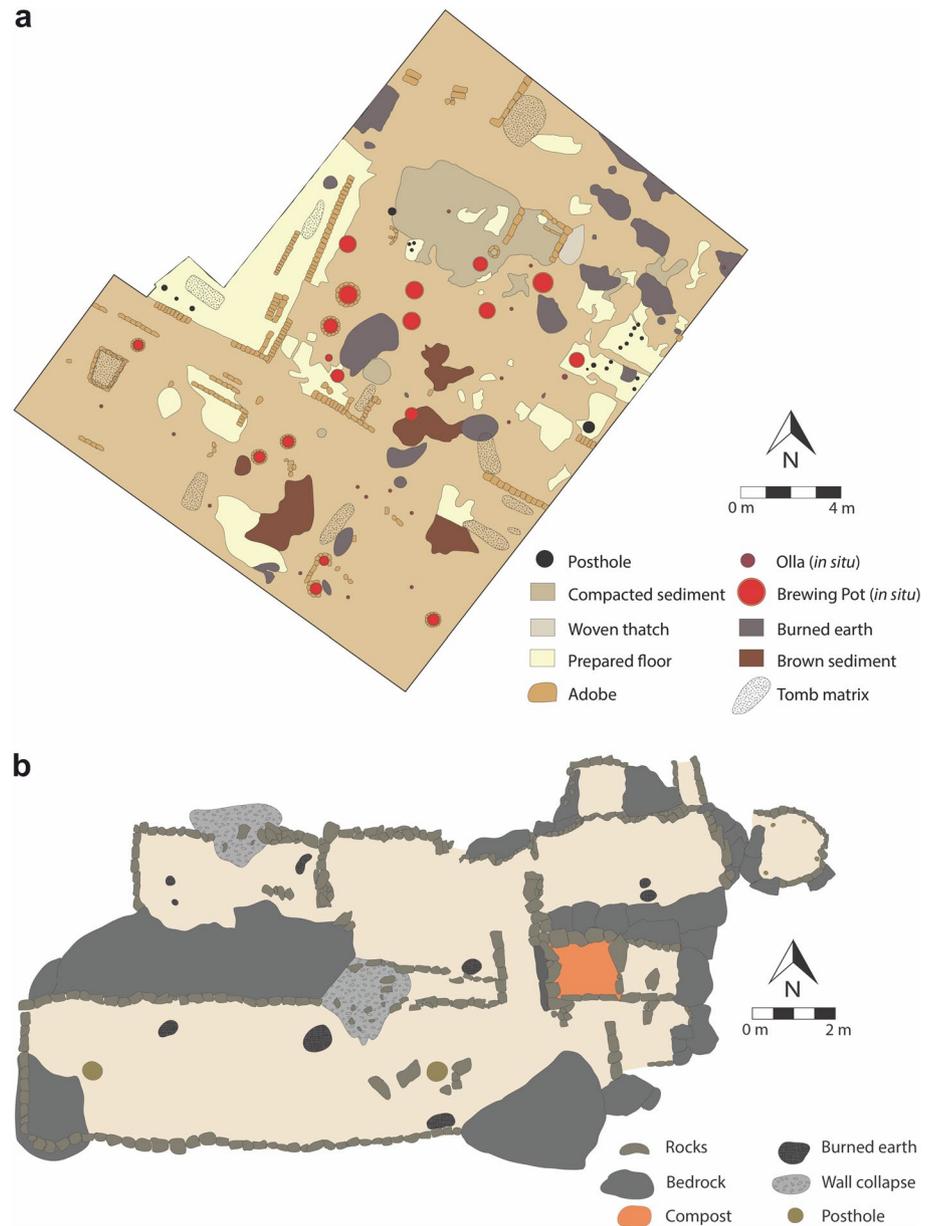
### Archaeological context

The macrobotanical remains that were examined in this study are connected with the Moche people(s), which comprised a complex political organization stretching up and down the northern Peruvian desert coast from AD 100–800 (Benson 1972; Bawden 1996; Quilter 2002; Castillo 2010; Chapdelaine 2011). These remnants of the seeds, fruits, and tubers of economically important plants were recovered from roughly contemporary Late Moche (AD 600–800) occupations at two sites located 4 km apart in the Jequetepeque valley (Fig. 1). The first site, San José de Moro, was a ceremonial centre and burial ground known for its intense association with ritual activities connected with the dead (Donnan and Castillo 1992; Castillo 1993, 2006, 2012). All of these remains were recovered from sediment samples collected during excavations in Area 35, a feast preparation zone with evidence for the large-scale brewing of *chicha* (maize beer) and the consumption of psychoactives (Fig. 2a; Prieto 2011; Chiou 2017). As a result, the macrobotanical remains are, for the most part, the by-products of food- and drink-related activities tied to feasting and funerary rituals.

**Fig. 1** Map of the Jequetepeque valley with the location of the study sites in red after Castillo (2012, p. 4, Fig. 1.2). Inset (A) shows the location of the study area in South America



**Fig. 2** **a** Plans of Area 35 in San José de Moro; **b** household 1 at Cerro Chepén



Nearly all of the plant remains recovered from San José de Moro were preserved through charring.

The second site is Cerro Chepén, a fortified settlement featuring a large defensive wall that separated elite residents from the commoner neighbourhoods (Rosas Rintel 2010). All of the remains from this site were recovered from sediment samples collected during excavations in Household 1, a commoner residence in Cerro Chepén's northern hillside community whose occupants were likely farming nearby fields (Fig. 2b; Chiou 2017). Contrary to the patterning of the plant evidence at San José de Moro, which reflected the untidiness and excess associated with feasts, the remains from Cerro Chepén are associated with discrete contexts where daily activities took place including plant processing,

cooking, and composting. The majority of the macrobotanical assemblage from Household 1 was recovered from a secondary deposit—a compost pit within the house—which contained a mixture of primarily camelid dung with the addition of guinea pig dung, plant remains, wood charcoal, and fish bones which, presumably, were meant to become fertilizer. Except for wood, most of the plant remains from Cerro Chepén were preserved through desiccation. Charred plant remains only dominated in a few contexts such as hearths.

All of the sediment samples from San José de Moro and Cerro Chepén were obtained using a systematic and judgmental sampling strategy that involved the collection of 10 l samples from every defined feature as well as 10 l

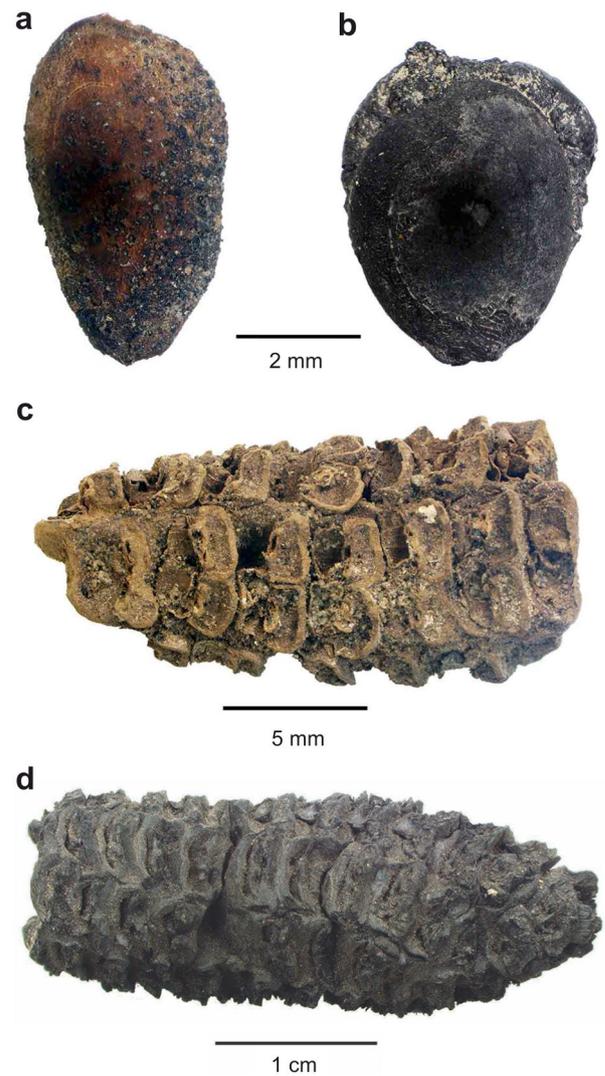
samples from a series of fixed points in the excavation area (Lennstrom and Hastorf 1992, 1995; Chiou 2017). A riffle-type sample splitter was then used to divide each sample into two theoretically “identical” sub-samples. These paired sub-samples were subjected to a dual-pronged processing strategy that involved either (A) water flotation using an SMAP-type mechanized flotation machine (Watson 1976) or (B) dry-sieving, a commonly employed method in desertic regions (Chiou 2017). The rationale for this approach was derived from a study by Chiou et al. (2013) that examined paired sets of samples from the Ica valley of Peru and suggested that while water flotation appears to favour charred remains and small seeds, dry-screening is optimal for desiccated remains, particularly those in the larger size fractions. These sub-samples were analysed, sorted, weighed, and identified to the most detailed taxonomic level possible in the McCown Archaeobotany Laboratory at the University of California, Berkeley, with the use of a low-powered stereomicroscope with 6.7× to 45× magnification.

### Sample description

To directly compare the isotopic measurements of charred and desiccated archaeobotanical remains, we selected 26 individual specimens from San José de Moro and Cerro Chepén that were identified from a range of economically important plant taxa. Most were recovered in both preservation states (Fig. 3). These taxa include *Lagenaria siceraria* (Molina) Standl. (bottle gourd), *Prosopis pallida* Kunth (algarrobo pálido), *Zea mays* L. (maize), *Manihot esculenta* Crantz (manioc), *Capsicum baccatum* Kunth (chili pepper), *Psidium guajava* L. (guava), *Cucurbita moschata* Duchesne ex Poiret (squash), *Phaseolus vulgaris* L. (common bean), *Erythroxylum* sp. (coca), *Gossypium barbadense* L. (cotton), and *Passiflora* sp. L. (passion fruit). Although the majority of our sampled remains were seeds, we also tested fragments of pericarp, endocarp, maize cobs, and fibre (cotton fluff). Most of the samples were well preserved with the exception of the desiccated remains from the compost pit, which exhibited some signs of partial decomposition, exposure to moisture, and in some cases, insect infestation (Chiou 2017).

### Sample preparation and isotopic analysis

Samples were treated with 10 ml of 0.5 M HCl at 80 °C for 30 min and then rinsed three times with Type I (resistivity = 18.2 MΩ cm) water (Vaiglova et al. 2014b). Samples were then air dried at 40 °C under normal atmosphere, homogenized by crushing with a glass stirring rod, and subsamples weighing  $550 \pm 115 \mu\text{g}$  were placed into 4.25 × 5 mm tin capsules. The carbon and nitrogen isotopic and elemental compositions were determined using



**Fig. 3** Examples of desiccated and charred remains used in this study, including: **a** desiccated *Prosopis pallida* (algarrobo) seed (1007.1) from Cerro Chepén; **b** charred *P. pallida* seed (1017.1) from Cerro Chepén; **c** desiccated *Zea mays* (maize) cob (1023.2) from Cerro Chepén; **d** charred *Z. mays* cob (1035B) from San José de Moro

a Costech 4010 elemental analyser coupled to a Delta V continuous flow isotope ratio mass spectrometer. Stable carbon and nitrogen isotope compositions were calibrated relative to VPDB and AIR using a two-point curve anchored by USGS40 ( $\delta^{13}\text{C} = -26.39 \pm 0.04\text{‰}$ ;  $\delta^{15}\text{N} = -4.52 \pm 0.06\text{‰}$ ) and USGS41a ( $\delta^{13}\text{C} = +36.55 \pm 0.08\text{‰}$ ;  $\delta^{15}\text{N} = +47.55 \pm 0.15\text{‰}$ ) (Qi et al. 2003, 2016). Analytical uncertainty was determined to be  $\pm 0.27\text{‰}$  for  $\delta^{13}\text{C}$  and  $\pm 0.3\text{‰}$  for  $\delta^{15}\text{N}$  following the method presented by Szpak et al. (2017); additional quality control and quality assurance data are presented in full in the Supplementary Material (ESM).

## Statistical analyses

To compare the elemental and isotopic compositions of the different types of plant remains, we first assessed the normality of each distribution using a Shapiro–Wilk test (Shapiro and Wilk 1965). Comparisons involving non-normal distributions were made using a Mann–Whitney  $U$  test (Mann and Whitney 1947). Those with normal distributions were compared using unpaired  $t$ -tests (Student 1908; Welch 1947). We also included the isotopic and elemental compositions of the charred and desiccated macrobotanical remains published by DeNiro and Hastorf (1985) in some statistical comparisons for comparative purposes. We examined the correlation between the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of the  $\text{C}_3$  plants analysed in this study using Spearman's  $\rho$ .

## Results

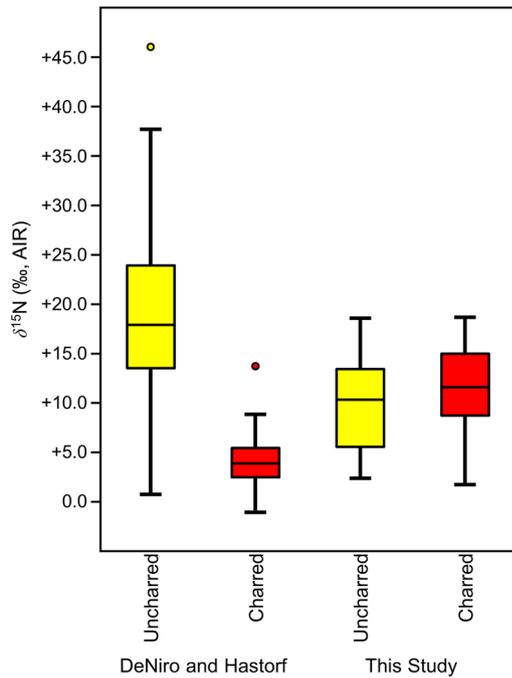
### Isotopic compositions of botanical remains

All of the samples analysed contained sufficient amounts of combustible carbon and nitrogen to produce  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  measurements. The stable isotope compositions for the analysed samples are presented in Table 1. These were in line with expectations for plants growing in this region. The nitrogen isotopic compositions of the charred and desiccated remains are presented in Fig. 4 along with the data from DeNiro and Hastorf (1985) for comparative purposes. The  $\delta^{15}\text{N}$  values of the charred and desiccated remains from San José de Moro and Cerro Chepén were not significantly different to each other ( $U = 66$ ,  $p = 0.48$ ). Conversely, the  $\delta^{15}\text{N}$  values presented by DeNiro and Hastorf (1985) for the desiccated remains from the coast

**Table 1** Isotopic and elemental compositions for all samples analysed in this study

Lab ID	Original ID	Site	Taxon	Part	$\delta^{13}\text{C}$ (‰, VPDB)	$\delta^{15}\text{N}$ (‰, AIR)	wt% C	wt% N	C:N <sub>Atomic</sub>
Charred remains									
564	1038A	San José de Moro	<i>Capsicum baccatum</i>	Seed	−23.94	+11.03	52.4	5.4	11.3
566	1021B	San José de Moro	<i>Erythroxylum</i> sp.	Endocarp	−25.09	+10.68	61.6	5.5	13.2
567	1076B	San José de Moro	<i>Gossypium barbadense</i>	Seed and fluff	−25.76	+13.15	64.7	4.3	17.7
544	1114B	San José de Moro	<i>Lagenaria siceraria</i>	Pericarp	−23.08	+18.63	57.9	4.9	13.9
556	1007B	San José de Moro	<i>Manihot esculenta</i>	Tuber	−23.88	+12.24	58.6	5.4	12.7
568	1021B	San José de Moro	<i>Passiflora</i> sp.	Seed	−26.68	+9.92	50.0	2.8	20.7
565	1076B	San José de Moro	<i>Phaseolus vulgaris</i>	Seed	−24.96	+8.34	52.3	6.4	9.6
549	1038A	San José de Moro	<i>Prosopis pallida</i>	Pod	−13.21	+11.59	49.5	5.1	11.3
546	1017.1	Cerro Chepén	<i>Prosopis pallida</i>	Seed	−23.39	+1.76	61.6	5.4	13.3
548	1095A	San José de Moro	<i>Prosopis pallida</i>	Seed	−23.65	+14.99	62.6	4.6	16.1
547	1038B	San José de Moro	<i>Prosopis pallida</i>	Seed <sup>a</sup>	−23.27	+14.66	61.2	5.1	14.0
561	1038A	San José de Moro	<i>Psidium guajava</i>	Seed	−26.21	+16.03	35.7	3.1	13.2
553	1035B	San José de Moro	<i>Zea mays</i>	Cob	−11.23	+6.44	62.5	1.9	37.6
555	1021B	San José de Moro	<i>Zea mays</i>	Cob	−11.17	+9.87	56.2	2.4	27.1
559	1029.2	Cerro Chepén	<i>Zea mays</i>	Kernel	−9.31	+4.77	59.1	5.9	11.6
551	1038B	San José de Moro	<i>Zea mays</i>	Kernel	−11.88	+13.03	54.4	4.7	13.6
Desiccated remains									
558	1023.1	Cerro Chepén	<i>Capsicum baccatum</i>	Seed	−23.71	+11.06	46.3	3.5	15.3
563	1023.7	Cerro Chepén	<i>Cucurbita moschata</i>	Seed	−24.95	+5.98	44.8	4.6	11.4
562	1023.6	Cerro Chepén	<i>Cucurbita moschata</i>	Seed	−22.23	+2.37	43.6	4.0	12.6
543	1023.1	Cerro Chepén	<i>Lagenaria siceraria</i>	Pericarp	−17.10	+12.57	26.4	2.8	11.1
557	1023.2	Cerro Chepén	<i>Manihot esculenta</i>	Tuber	−25.94	+4.64	45.0	5.6	9.3
550	1007.1	Cerro Chepén	<i>Prosopis pallida</i>	Seed	−21.14	+8.52	41.3	6.6	7.3
545	1023.1	Cerro Chepén	<i>Prosopis pallida</i>	Seed <sup>a</sup>	−20.49	+12.19	33.2	3.8	10.1
560	1029.7	Cerro Chepén	<i>Psidium guajava</i>	Seed	−27.44	+18.54	54.1	3.2	20.0
554	1023.2	Cerro Chepén	<i>Zea mays</i>	Cob	−12.39	+9.71	38.0	3.5	12.8
552	1023.1	Cerro Chepén	<i>Zea mays</i>	Cob	−12.22	+16.65	49.5	3.5	16.5

<sup>a</sup>Inside endocarp

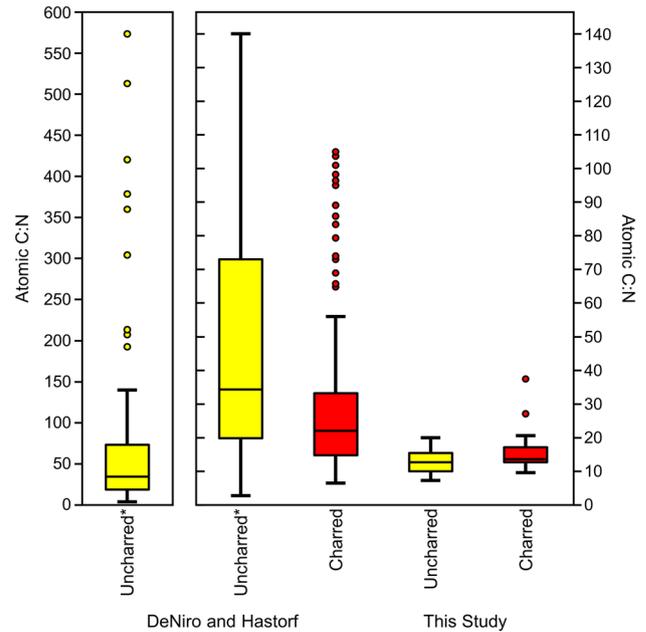


**Fig. 4** Nitrogen isotopic compositions for uncharred and charred botanical remains for this study (right) and the DeNiro and Hastorf (1985) study (left)

were significantly higher than the charred remains from the highlands ( $U = 326.5$ ,  $p < 0.001$ ).

### Elemental compositions of botanical remains

The elemental compositions (wt% C and wt% N) of the samples analysed in this study are presented in Table 1. The mean percentage weight of carbon (wt% C) for charred botanical remains was  $57.2 \pm 7.3\%$  while that for desiccated remains was  $42.2 \pm 5.8\%$ ; this difference was statistically significant (Mann–Whitney  $U$  test;  $U = 12$ ,  $p < 0.001$ ). The mean wt% N for charred botanical remains was  $4.5 \pm 1.3\%$  compared to  $4.2 \pm 1.1\%$  for desiccated remains, a difference that was not statistically significant (Mann–Whitney  $U$  test;  $U = 64$ ,  $p = 0.41$ ). The atomic C:N ratios for the samples analysed in this study are summarized in Fig. 5; the C:N ratios did not differ significantly between the two classes of remains ( $U = 55$ ,  $p = 0.20$ ). Conversely, the C:N ratios presented by DeNiro and Hastorf (1985) were significantly higher for desiccated plants (coastal) relative to charred plants (highlands) ( $U = 3,861$ ,  $p = 0.001$ ). They presented only C:N ratios and not elemental compositions, therefore we limit our comparisons between studies to atomic C:N ratios.



**Fig. 5** Atomic C:N ratios for charred and desiccated plants in this study (right) and the DeNiro and Hastorf (1985) study (left). \*Note that the uncharred plant data from the DeNiro and Hastorf (1985) study are depicted twice. The boxplot on the far left displays all of the data, including outliers while the other boxplot excludes these outliers to make it easier to compare the data generated in this study

## Discussion

### Charred vs. desiccated remains

Our findings do not corroborate the differences in  $\delta^{15}\text{N}$  or C:N between charred and desiccated remains observed by DeNiro and Hastorf (1985). There are several plausible and not mutually exclusive explanations for the difference between our results and theirs. First, the differences between the coastal and highland environments may have caused the  $\delta^{15}\text{N}$  values of the coastal plants to be higher. Plant  $\delta^{15}\text{N}$  values are strongly correlated with local water availability so that plants growing in dry locations have higher  $\delta^{15}\text{N}$  values than those growing in relatively moist locations (Austin and Vitousek 1998; Handley et al. 1999). The coast of Peru is extremely arid and wild plants growing in that region have higher  $\delta^{15}\text{N}$  values than those growing at higher altitudes (Szpak et al. 2013). The use of irrigation canals on the coast (Eling 1987) may have mitigated this  $^{15}\text{N}$  enrichment in plants driven by a lack of water availability, but this is speculative. Nonetheless, given the large variation in plant  $\delta^{15}\text{N}$  that exists among different environments (Amundson et al. 2003; Craine et al. 2009) the comparison of charred and desiccated plants from two very different ecological zones by DeNiro and Hastorf (1985) is probably a factor in the difference between our results and theirs.

The highest C:N ratio for a desiccated sample in our study was 20 while 74% of the desiccated plant remains presented by DeNiro and Hastorf (1985) had C:N ratios that were above 20 and 17% were above 100. The C:N ratios of plant remains are quite variable, but Fraser et al. (2013) found values around 20 and 10 for experimentally charred cereals and legumes, respectively, comparable to our results for San José de Moro and Cerro Chepén (Table 1). Dividing the DeNiro and Hastorf (1985) desiccated plant data according to C:N ratio, those with a C:N ratio below 20 had  $\delta^{15}\text{N}$  values of  $+13.9 \pm 7.9\text{‰}$ , while those with C:N ratios above 20 had  $\delta^{15}\text{N}$  values of  $+20.3 \pm 8.1\text{‰}$ ; this difference was statistically significant ( $t=3.33$ ,  $p=0.001$ ). Most of the samples with especially high C:N ratios likely had very small amounts of nitrogen rather than extremely high amounts of carbon; note that the wt% C and wt% N were not published, only the C:N ratios. The degradation of this nitrogen would have been associated with the preferential loss of  $^{14}\text{N}$ , leaving the residual nitrogen enriched in  $^{15}\text{N}$  (Mariotti et al. 1981); higher  $\delta^{15}\text{N}$  values in decomposed plants relative to fresh ones have been observed experimentally (Tahmasebi et al. 2018). The fact that some, but not all, of DeNiro and Hastorf's samples had unusually high  $\delta^{15}\text{N}$  values may simply reflect the large amount of variation in the original  $\delta^{15}\text{N}$  values of the plants at the time of burial or the highly variable amount of nitrogen that was lost during decomposition across different sites. Regardless, a strong negative correlation between the amount of nitrogen and its  $\delta^{15}\text{N}$  value should exist (Mariotti et al. 1981) and this may help to explain the very high  $\delta^{15}\text{N}$  values in the DeNiro and Hastorf (1985) dataset, particularly for plants with high C:N ratios.

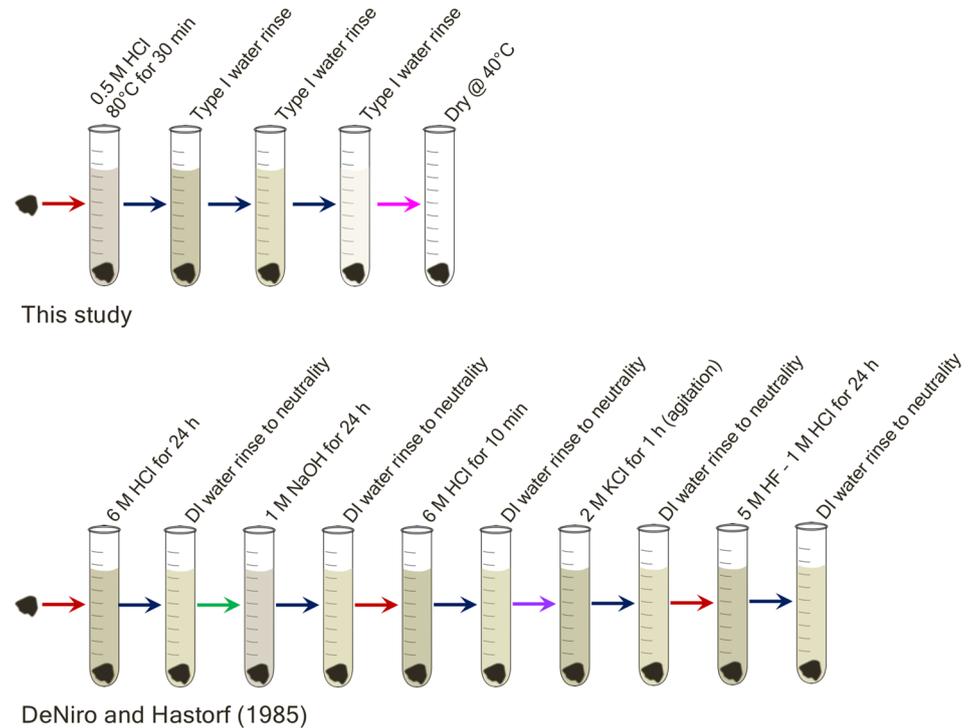
The  $\delta^{15}\text{N}$  values of samples with very low N contents are difficult to accurately or precisely measure. For example, bone collagen samples with very low %N may produce  $\delta^{15}\text{N}$  values that are either impossibly high or impossibly low and not reproducible. Simply increasing the sample size in an attempt to generate an amount of gas that is comparable to the reference gas does not mitigate this problem (Szpak, personal observation). This is why the elemental concentrations (wt% C and wt% N) of bone collagen are considered to be robust indicators for the preservation of the original isotopic composition of the sample (Ambrose 1990). The same may be true for organic matter derived from macrobotanical remains, with those producing unusually low nitrogen concentrations being characterized by unreliable and usually too high  $\delta^{15}\text{N}$  values, because of the preferential loss of  $^{14}\text{N}$  during degradation. A possible quality control metric for macrobotanical  $\delta^{15}\text{N}$  values may be to plot the C:N ratios against the  $\delta^{15}\text{N}$  values for a given taxon and if a strong correlation exists, this may signal alteration of the original isotopic composition in the burial environment, most likely for those samples with

high  $\delta^{15}\text{N}$  and C:N ratios. We would stress, however, that experimental studies are required to explore the relationship of plant  $\delta^{15}\text{N}$  values, as degradation occurs in the burial environment.

The sample pre-treatment protocol used by DeNiro and Hastorf (1985) was markedly different than the one employed in this study and involved lengthier treatments with stronger chemicals (Fig. 6). Vaiglova et al. (2014b) found that treatments with more concentrated acids (such as 6 M rather than 0.5 M HCl) could alter the  $\delta^{15}\text{N}$  values of charred plant remains. They did not test the effect of different sample pre-treatments on desiccated plant remains and of the range of chemical pre-treatments used by DeNiro and Hastorf (1985), only the first three chemical treatments were tested. It is, therefore, unknown how treatment with HF or KCl would influence the  $\delta^{15}\text{N}$  values of charred or desiccated plant remains, if at all. Nonetheless, we must acknowledge the possibility that one of the reasons that our results differ from those of DeNiro and Hastorf (1985) with respect to charred vs. desiccated plants could be because of differences in sample preparation techniques.

A substantial body of research now exists demonstrating that charred plant remains from archaeological contexts produce reliable stable isotope measurements (Kanstrup et al. 2012; Fraser et al. 2013; Styring et al. 2013; Vaiglova et al. 2014b; Nitsch et al. 2015, 2018; Brinkkemper et al. 2018). The fact that both the  $\delta^{15}\text{N}$  values and C:N ratios were indistinguishable between the two classes of remains at Cerro Chepén and San José de Moro implies that both charred and desiccated remains can produce isotopic measurements that reflect the original isotopic compositions of the plants. Our results demonstrate that the findings of DeNiro and Hastorf (1985) do not provide adequate justification to dismiss isotopic measurements of desiccated botanical remains. Issues with the experimental design (comparing charred materials from the highlands with desiccated materials from the coast), sample preparation (aggressive chemical pre-treatment), and mass spectrometry (analysis of samples with extremely low amounts of nitrogen) may have contributed to the differences that they observed. At the very least, the results of our study demonstrate the need to further explore the usefulness of uncharred archaeological plant remains for isotopic analysis. Although not based on comparisons of charred and uncharred botanical remains, Metcalfe and Mead (2019) arrived at similar conclusions regarding plant  $\delta^{13}\text{C}$  measurements, citing isotopic differences among plant parts, comparisons with herbivore tissue isotopic compositions of similar date, and differences in  $\delta^{13}\text{C}$  between CAM and  $\text{C}_4$  plants. We would encourage additional studies to be conducted to verify the results presented here with larger numbers of samples, a larger range of taxa, and materials from other regions where both classes of macrobotanical remains may co-occur.

**Fig. 6** Comparison of the sample pre-treatments used in this study and by DeNiro and Hastorf (1985)

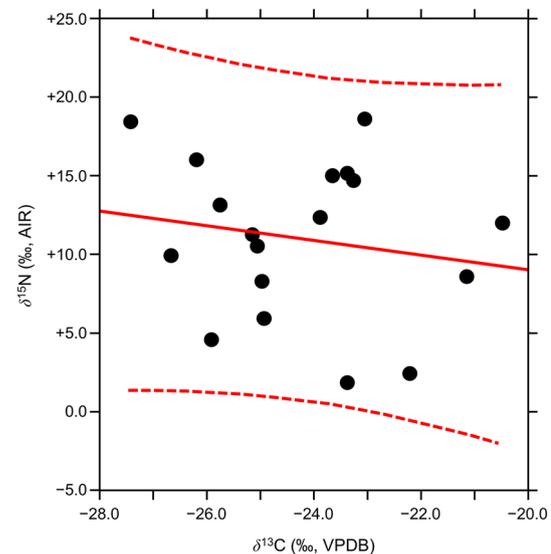


### Implications for agricultural intensification

Because of the limited sample size, we treat these results as a pilot study that should be interpreted with caution. Variation in the isotopic compositions of plants grown in the same field has been observed, especially for fertilized fields (Szpak et al. 2012b, 2019a; Fraser et al. 2013). Moreover, there may be isotopic variation within a plant structure, for example among beans within a single pod (Bogaard et al. 2007), although the magnitude of this variation is relatively small (Szpak et al. 2014a). Because of these sources of variation, much larger sample sizes are necessary to make firm interpretations of agricultural intensification.

The  $\delta^{15}\text{N}$  values of both the charred and desiccated remains were relatively high but not uniformly so (Fig. 4), which could be the product of variable rates of fertilization or water stress, as both of these result in plants with tissues relatively enriched in  $^{15}\text{N}$  (Szpak 2014). One important difference, however, is that plant tissue  $\delta^{13}\text{C}$  values are strongly influenced by water availability (Condon et al. 1992; Stewart et al. 1995; Murphy and Bowman 2009), while fertilization has no effect (Szpak et al. 2012a, b, 2014a). Therefore, if the comparatively high  $\delta^{15}\text{N}$  values in this dataset are the product of water-stressed plants, we would expect a positive correlation between plant  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  for  $\text{C}_3$  plants (Swap et al. 2004); the  $\delta^{13}\text{C}$  values of  $\text{C}_4$  plants are not impacted by water availability (Schulze et al. 1996; Swap et al. 2004). Conversely, a lack of any correlation between  $\text{C}_3$  plant  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  would be consistent with the high  $\delta^{15}\text{N}$  values

being caused by the application of some type of fertilizer. For the  $\text{C}_3$  plants analysed in this study, the correlation between  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  was neither strong nor statistically significant (Fig. 7; Spearman's  $\rho = -0.11$ ,  $p = 0.67$ ), suggesting that the high  $\delta^{15}\text{N}$  values are the result of fertilization.



**Fig. 7** Comparison of the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of  $\text{C}_3$  plants analysed in this study. The solid line is the line of best fit for the linear regression and the dashed lines represent the lower and upper limits of the 95% confidence interval

On the basis of these data it is not possible to identify the type of fertilizer or the quantity applied, but camelid dung and seabird guano are likely candidates. Guano can cause plants to have extremely high  $\delta^{15}\text{N}$  values ( $> 30\text{‰}$ ), although the degree of  $^{15}\text{N}$  enrichment varies according to the rate of application (Szpak et al. 2012a). It is therefore plausible that a moderate amount of seabird guano could have been applied to the crops to produce the relatively high  $\delta^{15}\text{N}$  values that were found in our samples. Sulphur isotopic compositions could help to resolve this issue, as camelid dung should not produce unusually high  $\delta^{34}\text{S}$  values in fertilized plants but seabird guano does (Szpak et al. 2019a).

Relative to guano, camelid dung appears to produce a more modest increase in plant  $\delta^{15}\text{N}$  values (by 2–4‰), comparable to other domestic herbivores (Szpak 2014). We do not know, however, what the  $\delta^{15}\text{N}$  values of the camelids that may have produced this dung would have been because there are no published  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  values for camelids from either Cerro Chepén or San José de Moro. Nonetheless, the mean bone collagen  $\delta^{15}\text{N}$  values for camelids from other sites on the north coast of Peru range between +6.6 and +8.3‰ (Szpak et al. 2014b, 2016b, 2019b; Dufour et al. 2018). Sponheimer et al. (2003) found that the  $\delta^{15}\text{N}$  of llama faeces was 2.9‰ higher than that of the diet. Given that bone collagen values are 3–5‰ higher than that of the diet (Ambrose 2000; Post 2002), this would imply that the  $\delta^{15}\text{N}$  of faeces would be 0–2‰ lower than the bone collagen of the animals that produced it. Therefore, if the camelids that were present at Cerro Chepén or San José de Moro had tissue  $\delta^{15}\text{N}$  values comparable to those of others from the north coast of Peru, the  $\delta^{15}\text{N}$  of the dung of these camelids could be expected to be between +4.6 and +8.3‰. There is, however, a large difference between the  $\delta^{15}\text{N}$  of fresh and composted manures. During composting, the volatilization of  $\text{NH}_4^+$  to gaseous  $\text{NH}_3$  occurs and there is a large fractionation ( $\sim 40\text{‰}$ ) associated with this process (Kreitler 1975; Mizutani et al. 1985). The  $\delta^{15}\text{N}$  of the residual manure can therefore become much higher and consequently crops fertilized with composted manure can also have much higher  $\delta^{15}\text{N}$  values than those fertilized with raw, fresh manure (Choi et al. 2017). Nitrogen isotope compositions around +20‰ have been recorded for crops fertilized with composted manure (Choi et al. 2003; Yun and Ro 2009), representing the upper end of the range of values that have been observed for animal manures, excluding seabird guano (Szpak 2014). This is noteworthy because evidence for the composting of camelid dung exists at Cerro Chepén (Chiou 2017), the purpose of which was likely to make manure to be applied to fields. We cannot definitively rule out the possibility that seabird guano may have been included in the compost pit, but given the low organic and high mineralized nitrogen contents of guano (Hutchinson 1950), composting would not improve the quality of this

fertilizer. Therefore, we suggest that composted camelid dung was applied to fields to increase soil fertility. While it is difficult to assess how widespread a practice this was, it does imply that camelids would have been locally present in large enough numbers to produce a sufficient quantity of dung to fertilize fields. Given the growing body of evidence supporting the presence of populations of camelids in the coastal river valleys of Peru (Shimada and Shimada 1985; Bonavia 2008; Dufour et al. 2014, 2018; Szpak et al. 2014b, 2015, 2016a, b, 2018, 2019b; Tomczyk et al. 2019), it seems unlikely that this practice of fertilizing crops with camelid dung would have been limited to the Jequetepeque valley during the Moche occupation. If the practice of manuring with camelid dung was reasonably widespread in the coastal river valleys, this implies a substantial level of integration of the agricultural and pastoral economies. Additional isotopic analyses of macrobotanical remains from a range of sites are required to test this hypothesis.

## Conclusions

We have presented  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for charred and desiccated plant remains from two archaeological sites in the Jequetepeque valley, northern Peru. Although there were differences in the elemental compositions of the two classes of remains, their nitrogen isotopic compositions did not differ significantly. This result implies that in addition to charred plant remains, desiccated plant remains can also produce isotopic measurements that reflect the original isotopic compositions of the living plants. This opens up the possibility of obtaining isotopic data from a wider range of plant taxa and tissues than would be possible by relying solely on charred specimens. Further studies examining both charred and desiccated plant remains from the same contexts are, however, required to validate the results presented here before large-scale sampling efforts integrating variable proportions of both classes of remains begin to examine issues related to agricultural intensification.

The  $\delta^{15}\text{N}$  values of the charred and desiccated plant remains from San José de Moro and Cerro Chepén were relatively high ( $+11.2 \pm 4.4\text{‰}$  for charred remains and  $+10.1 \pm 5.1\text{‰}$  for desiccated remains), suggesting that the inhabitants were making use of animal manure to improve soil fertility. On the basis of the presence of a compost pit containing substantial quantities of camelid dung at Cerro Chepén we suggest that composted camelid manure is the most likely source of the high  $\delta^{15}\text{N}$  values in the plant remains.

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