Carbon and Nitrogen Isotopic Survey of Northern Peruvian Plants: Baselines for Paleodietary and Paleoecological Studies

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Abstract

The development of isotopic baselines for comparison with paleodietary data is crucial, but often overlooked. We review the factors affecting the carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic compositions of plants, with a special focus on the carbon and nitrogen isotopic compositions of twelve different species of cultivated plants ($n=91$) and 139 wild plant species collected in northern Peru. The cultivated plants were collected from nineteen local markets. The mean $\delta^{13}C$ value for maize (grain) was $-11.8\pm0.4\%$ ($n=27$). Leguminous cultigens (beans, Andean lupin) were characterized by significantly lower $\delta^{15}N$ values and significantly higher %N than non-leguminous cultigens. Wild plants from thirteen sites were collected in the Moche River Valley area between sea level and ~4,000 meters above sea level (masl). These sites were associated with mean annual precipitation ranging from 0 to 710 mm. Plants growing at low altitude sites receiving low amounts of precipitation were characterized by higher $\delta^{15}N$ values than plants growing at higher altitudes and receiving higher amounts of precipitation, although this trend dissipated when altitude was ~2,000 masl and MAP was ~400 mm. For C3 plants, foliar $\delta^{13}C$ was positively correlated with altitude and precipitation. This suggests that the influence of altitude may overshadow the influence of water availability on foliar $\delta^{13}C$ values at this scale.

Introduction

Stable isotope analysis is an important tool for reconstructing the diet, local environmental conditions, migration, and health of prehistoric human and animal populations. This method is useful because the carbon and nitrogen isotopic compositions of consumer tissues are directly related to the carbon and nitrogen isotopic compositions of the foods consumed [1,2], after accounting for the trophic level enrichments of $^{13}C$ and $^{15}N$ for any particular tissue [3,4].

In all cases, interpretations of isotopic data depend on a thorough understanding of the range and variation in isotopic compositions of source materials [5]. For instance, studies of animal migrations using oxygen and hydrogen isotopic analyses require a thorough understanding of the spatial variation in surface water and precipitation isotopic compositions [6], and in that avenue of research, there has generally been an emphasis on establishing good baselines. With respect to diet and local environmental conditions, the interpretation of isotopic data (typically the carbon and nitrogen isotopic composition of bone or tooth collagen) depends upon a thorough knowledge of the range and variation in isotopic compositions of foods that may have been consumed. Although several authors have attempted to develop such isotopic baselines for dietary reconstruction [7–10], these studies have typically focused on vertebrate fauna.

Despite the fact that plants are known to be characterized by extremely variable carbon and nitrogen isotopic compositions [11,12], few studies have attempted to systematically document this variability in floral resources at a regional scale using an intensive sampling program, although there are exceptions [13–15]. This is problematic, particularly in light of the development and refinement of new techniques (e.g. isotopic analysis of individual amino acids), which will increase the resolution with which isotopic data can be interpreted. If isotopic baselines continue to be given marginal status, the power of new methodological advancements will never be fully realized.

With respect to the Andean region of South America, the isotopic composition of plants is very poorly studied, both from ecological and paleodietary perspectives. The most comprehensive study of the latter type was conducted by Tieszen and Chapman [14] who analyzed the carbon and nitrogen isotopic compositions of plants collected along an altitudinal transect (~0 to 4,400 masl) following the Lluta River in northern Chile. Ehleringer et al. [16] presented $\delta^{13}C$ values for plants along a more limited altitudinal transect in Chile (Atacama Desert). A number of other studies...
have provided isotopic data on a much more limited scale from various sites in Argentina [17–21], Chile [22–24], Bolivia [25,26], Ecuador [26], Colombia [26], and Peru [26–30].

The number of carbon and nitrogen isotopic studies in the Andean region has increased dramatically in the last ten years, facilitated by outstanding organic preservation in many areas. The majority of these studies have been conducted in Peru [31–42] and Argentina [18–21,43–47]. With respect to northern Peru in particular, a comparatively small number of isotopic data have been published [40,48,49], although this will certainly rise in coming years as biological materials from several understudied polities (e.g. Virú, Moche, Chimú) in the region are subjected to isotopic analysis.

The purpose of this study is to systematically examine the carbon and nitrogen isotopic compositions of plants from the Moche River Valley in northern Peru collected at various altitudes from the coast to the highlands. These data provide a robust baseline for paleodietary, paleoecological, and related investigations in northern Peru that will utilize the carbon and nitrogen isotopic compositions of consumer tissues.

Study Area

The Andes are an area of marked environmental complexity and diversity. This diversity is driven largely by variation in altitude (Figure 1). As one proceeds from the Pacific coast to the upper limits of the Andes, mean daily temperature declines, typically by ~5°C per 1,000 m [50], and mean annual precipitation increases (Figure 2). The eastern slope of the Andes, which connects to the Amazon basin, is environmentally very different from the western slope. Because this study deals exclusively with the western slope, the eastern slope is not discussed further. Many authors have addressed the environment of the central Andes [51–58], hence only a brief review is necessary here.
The coastal region of Peru is dominated by the hyper-arid Peruvian desert. Cool sea-surface temperatures created by the northward flowing Peruvian Current, combined with a subtropical anticyclone, create remarkably stable and relatively mild temperatures along the roughly 2,000 km north-south extent of the Peruvian desert [55]. The phytogeography of the coastal region of Peru is fairly homogenous, although the composition of the vegetation varies in accordance with local topography [59]. Except in El Niño years, precipitation is extremely low or non-existent along much of the Peruvian coast, but in areas where topography is steep close to the coast, a fog zone forms (typically between 600 and 900 masl), which allows for the development of ephemeral plant communities (lomas) [60–62]. Aside from these lomas, riparian vegetation grows in the relatively lush river valleys that cut into the Andes, although the vast majority of this land is cultivated. Thickets of the leguminous algarroba tree regularly occur at low altitudes, and it is generally believed that much more extensive forests of these trees existed in the past [63,64]. The coastal zone usually ends where the oceanic influence becomes minimal, typically about 1,000 masl [58].

Immediately above the area of oceanic influence and up to an altitude of ~1,800 m, the environment is cooler, although generally similar, in comparison to the coastal zone. Although mean annual precipitation increases, this zone can still be characterized as dry, with most locations receiving less than 400 mm of annual precipitation. In some circumstances, lomas may form within this zone [52], although this is not common. In the Moche River Valley of northern Peru, the vegetation is dominated by xerophytic scrub vegetation from 500 to 1,800 masl, and transitions to thorny steppe vegetation between 1,800 and 2,000 masl. Again, the area is still characterized by relatively low annual precipitation, although water availability is greater close to

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**Figure 2. Extrapolated mean annual precipitation for study area.** Mean annual precipitation data from 493 monitoring stations in Peru [218] were extrapolated using the natural neighbor method in ArcMap (ArcGIS 10.0, ESRI). doi:10.1371/journal.pone.0053763.g002
major watercourses and other ground water sources. Ascending further, mean annual precipitation increases, and average daily temperature decreases. Night frost begins to occur. Vegetation is largely dominated by low-growing shrubs, herbs, and grasses, as well as open stands of some tree species (Acacia, Polygala) [56]. Pastures dominated by dense bunchgrasses occur in moistier areas.

**Natural Variation in Plant Carbon Isotopic Composition**

**Photosynthetic pathway and taxonomy.** The most salient mechanism influencing the carbon isotopic composition (δ13C) of terrestrial plants is the photosynthetic pathway utilized. Plants that fix carbon using the C3 pathway (Calvin cycle) are characterized by lower δ13C values (ca. −26 ‰) than plants utilizing the C4 (Hatch–Slack) pathway (ca. −12 ‰) [65,66]. This is because carbon isotope discrimination (Δ13C) is smaller in C4 plants than in C3 plants. In other words, C4 plants discriminate more strongly against the heavier isotope (13C) than C3 plants. The vast majority of C3 plants are tropical grasses, the most significant of which in New World archaeological contexts is maize (Zea mays), but also amaranth (Amaranthus caudatus). With respect to human diet, most wild C4 plants are not significant, and thus a large body of research has focused on assessing and quantifying the contribution of C4 cultigens (mostly maize, but also millet) to the diet [67]. Some desert plants and succulents exhibit carbon isotopic compositions that are intermediate between C4 and C3 plants. Referred to as CAM (Crassulacean acid metabolism) plants, these species fix carbon in a manner analogous to C4 plants overnight, but utilize the C3 photosynthetic pathway during the afternoon [68].

Additional plant groups that are not readily assigned into the aforementioned categories include mosses and lichens. Mosses, which are non-vascular plants, utilize the C4 photosynthetic pathway [69,70], but are distinct from vascular plants in that they lack stomata and CO2 availability is influenced primarily by the thickness of the water film accumulated on the leaves. Lichens are composite organisms, consisting of two parts: a mycobiont (fungi) and photobiont or phycobiont (algae). The carbon isotopic composition of lichens is determined largely by the type of photobiont involved. Lichens with green algae as the photobiont exhibit a wide range of carbon isotopic compositions (−35 to −17 ‰), while lichens with cyanobacteria as the photobiont tend to have higher, and a more restricted range of carbon isotopic compositions (−23 to −14 ‰) [71–73].

**Environmental factors affecting plant δ13C.** Aside from the differences in carbon isotopic composition resulting from variable carbon fixation, a number of environmental factors have also been demonstrated to influence the carbon isotopic composition of plant tissues. For example, low-growing plants under dense forest cover tend to exhibit lower δ13C values relative to canopy plants and plants growing in more open environments. Often referred to as the ‘canopy effect’, this is attributed to relatively 13C-depleted CO2 in the understory due to the utilization of recycled CO2 [74–78], and/or lower irradiance and higher [CO2] relative to the canopy [79,80]. The magnitude of differences in plant carbon isotopic composition observed due to the canopy effect typically range between 2 and 5 ‰ [81]. It has been postulated that the canopy effect significantly impacts the carbon isotopic composition of consumer tissues and thus reflects the use of closed and open habitats [82–84]. None of the sites sampled in this study were characterized by sufficiently dense forest for a canopy effect to have been significant.

Water availability has been observed to be negatively correlated with the carbon isotopic composition of plants [85–91]. In most instances, these effects are limited to C3 plants, with most studies finding little or no correlation between rainfall and/or water availability and plant δ13C for C4 plants [86,92]. Murphy and Bowman [87] found a positive correlation between rainfall and C4 plant δ13C over a continental (Australia) rainfall gradient, although this relationship is atypical. It is believed that the relationship between aridity and plant δ13C is caused by increased stomatal closure when water availability is low, which is accompanied by decreased discrimination against 13C during photosynthesis and, in turn, comparatively less negative δ13C values [93,94].

Soil salinity has also been demonstrated to influence plant δ13C values. In a manner somewhat analogous to drought stress, salt stress induces increased stomatal closure, and therefore reduces discrimination against 13C by the plant [95]. A number of studies have observed this relationship, which occurs in both halophytic (salt-tolerant) [96,97] and non-halophytic species [98,99].

A number of studies have found elevated gradients in plant carbon isotopic composition. Generally, foliar δ13C values have been found to increase with increasing altitude [88,100,101]. It is important to point out, however, that the majority of these studies have examined the isotopic composition of a single species or a small number of species over an elevational gradient of ~1,000 m. The exact mechanism responsible for the relationship between plant δ13C and altitude is not entirely clear. Some have suggested exceptionally high carboxylation rates relative to stomatal conductance [102,103] and/or high carbon fixation efficiency [104] for plants growing at high altitudes, resulting in decreased discrimination against 13C. A very strong positive correlation has been observed between altitude and leaf mass per unit area [100,101], which is thought to be instrumental in increasing carboxylation capacity.

Irradiance has also been shown to influence foliar δ13C values, with higher irradiance being associated with less negative δ13C values in leaves. Such variation can occur within a single plant (usually trees), and even along a single branch, with leaves growing in interior, shaded areas having lower δ13C values than leaves growing in exterior, exposed areas [105,106]. These differences in δ13C associated with irradiance have been attributed to differences in intercellular CO2 concentration [94].

**Intraplant and temporal variation in plant δ13C.** Carbon isotopic composition is not necessarily equal among different plant parts. Numerous studies have observed variation in the δ13C values of leaves, stems, roots, and other tissues [107–109]. The vast majority of studies examining the carbon isotopic compositions of multiple plant tissues have found that leaves are slightly depleted of 13C relative to non-photosynthetic tissues, typically by 2 to 4 ‰ [108,110,111]. These differences are only consistent among C3 plants, with C4 plants often showing little variation between leaves and non-photosynthetic tissues, or leaves with relatively high δ13C values in some cases [107,108]. There are several potential variables contributing to intraplant variation in tissue δ13C. First, different tissues may contain variable proportions of molecules that are relatively enriched or depleted of 13C compared to total organic matter. Most notably, lipids [112] and lignin [113] are known to be characterized by relatively low δ13C values, while the opposite is true for cellulose, sugars, and starches [114]. Because some studies have found significant differences in the δ13C of specific compounds (e.g. cellulose, sucrose) between different plant parts [110,111], it is thought that additional mechanisms are responsible for the observed patterns in intraplant δ13C variation. Damesin and Lelarge [110] suggest that some discrimination occurs during the translocation of sugars, particularly when certain plasma membrane proteins are involved in phloem transport. Potential mechanisms causing intraplant variation in δ13C are treated at length by Cernusak et al. [109].
In addition to variation among plant parts, a number of studies have found variation in $\delta^{13}C$ within plant parts, over time. Specifically, emerging leaves, which are not yet photosynthetic and therefore more closely resemble other non-photosynthetic or heterotrophic plants parts, tend to have less negative $\delta^{13}C$ values (by about 1 to 3 $\%$o) relative to fully emerged, photosynthetic leaves [91,110,111]. Products assimilated via photosynthesis will tend to have lower $\delta^{13}C$ values than those acquired heterotrophically, and this is likely partially responsible for the decrease in leaf $\delta^{13}C$ over time [115].

Marine plants. For the purpose of this paper, 'marine plants' refers specifically to macroalgae, or plants that are typically classified as kelps, seaweeds, and seagrasses. One of the most commonly reported distinctions in carbon isotopic composition is that marine plants tend to have higher $\delta^{13}C$ values than terrestrial animals, except in cases where C3 plants dominate the diet of the latter. While this distinction holds in the vast majority of circumstances [8,116,117], the same relationship is not necessarily true for marine and terrestrial plants.

Marine plants are characterized by a high degree of variability in carbon isotopic composition. Figure 3 presents the carbon isotopic compositions for the four major classes of marine macroalgae. In general, marine plants are characterized by carbon isotopic compositions that are intermediate in comparison to terrestrial C3 and C4 plants, with two notable exceptions. Seagrasses (Zostera sp.), have extremely high $\delta^{13}C$ values, typically higher than most terrestrial C4 plants (Figure 3d). There is evidence to suggest C4 photosynthetic activity in a few species of marine algae [118], but the comparatively high $\delta^{13}C$ values observed in many species, including seagrasses, cannot typically be explained in this way [119]. The variable use of dissolved CO2 and HCO3− in marine algae [118], but the comparatively high $\delta^{13}C$ values observed in many species, including seagrasses, cannot typically be explained in this way [119]. The variable use of dissolved CO2 and HCO3− in marine algae [118], but the comparatively high $\delta^{13}C$ values observed in many species, including seagrasses, cannot typically be explained in this way [119].

Some red algae (Floridiophyceae) are characterized by consistently very low $\delta^{13}C$ values ($< -30 \%$o). In general, the brown algae (kelps) have been noted to contribute significantly to nearshore ecosystems in terms of secondary production, with numerous studies examining the relative contributions of offshore phytoplankton and nearshore macroalgae [128].

Natural Variation in Plant Nitrogen Isotopic Composition

Nitrogen Source. Unlike carbon, which is obtained by plants as atmospheric CO2, nitrogen is actively taken up from the soil in the vast majority of cases. The two most important nitrogenous species utilized by plants are nitrate (NO3−) and ammonium (NH4+). In general, nitrate is the most abundant form of mineralized nitrogen available to plants, but in some instances, such as waterlogged or acidic soils, ammonium may predominate [129,130]. Additionally, some plants rely, at least to some extent, on atmospheric N2, which is obtained by symbiotic bacteria residing in root nodules (rhizobia) [131]. Plants may also take up organic nitrogen (e.g. free amino acids) from the soil [132], although the relative importance of these processes is not well understood and relatively poorly documented [133,134]. The extent to which plants rely on these N sources is significant because they may have distinct nitrogen isotopic compositions due to different nitrogen assimilation processes and the different nitrogen pools they utilize.

There are two important aspects of variation in N source pertinent to the present study. The first relates to N2-fixation by plants (mostly members of Fabaceae), which are common in both wild and domestic contexts in many parts of the central Andes. Plants that utilize significant amounts of atmospheric N2 are characterized by lower $\delta^{15}N$ values, typically ~0 $\%$o [27,138–140]. These plants acquire such compositions because the $\delta^{15}N$ of atmospheric N2 is ~−8 $\%$o [141] and the assimilation of N from N2-fixation is not associated with significant fractionation of $\delta^{15}N$ [130–140]. By comparison, soil NO3− and NH4+ tend to have $\delta^{15}N$ values >0 $\%$o [142], and non N2-fixing plants have $\delta^{15}N$ values that tend to be >0 $\%$, although these are highly variable for a number of reasons as discussed in more detail below.

The second potentially significant source-related cause of plant $\delta^{15}N$ variation is the uptake of fertilizer-derived N by plants. Animal fertilizers are characterized by extremely variable $\delta^{15}N$ values depending on the relative proportions of N-bearing species in the fertilizer (e.g. urea, uric acid, ammonium, organic matter) [143]. Manures consisting primarily of solid waste derived from terrestrial herbivores tend to have $\delta^{15}N$ values between 2 and 8 $\%$o [144], while those that contain a mix of solid and liquid waste (slurry fertilizers) tend to have higher $\delta^{15}N$ values, often between 6 and 15 $\%$o [145,146]. The highest $\delta^{15}N$ values for animal fertilizers (>25 $\%$o) have been recorded for seabird guano [143,147], which consists primarily of uric acid and is subject to significant NH4+ volatilization. The addition of animal fertilizer N to the soil therefore adds an N source with an isotopic composition that is usually enriched in $\delta^{15}N$ relative to endogenous soil N. This results in higher $\delta^{15}N$ values for plants growing in soils fertilized with animal waste than those plants growing in unfertilized soil or soils fertilized with chemical fertilizers [143,145–147].

Animal-derived N may be delivered to plants by means other than purposeful application of manures. Several studies have documented that the addition of N from animal carcasses (salmon in particular) provide substantial quantities of N taken up by plants. These plants tend to be characterized by relatively high $\delta^{15}N$ values [140,149]. Increased grazing intensity has also been suggested to influence plant $\delta^{15}N$ values due to the concentrated addition of animal waste, but studies have produced conflicting results, with some finding grazing to: increase plant $\delta^{15}N$ values [150,151], decrease plant $\delta^{15}N$ values [152,153], have little or no impact on plant $\delta^{15}N$ values [154,155], or increase $\delta^{15}N$ in plant roots, but decrease $\delta^{15}N$ in shoots [156].

Taxonomic variation. Strong distinctions in plant $\delta^{15}N$ have been related to mycorrhizal (fungal) associations [12,157,150]. In some ecosystems, particularly those at high latitudes characterized by soils with low N content, this facilitates the distinction between plant functional types – trees, shrubs, and grasses [159–161]. In a global survey of foliar $\delta^{15}N$ values, Craine et al. [12] found significant differences in plant $\delta^{15}N$ on the basis of mycorrhizal associations, with the following patterns (numbers in parentheses are differences relative to non-mycorrhizal plants): ericoid (~2 $\%$), ectomycorrhizal (~3.2 $\%$), arbuscular (~5.9 $\%$). The comparatively low $\delta^{15}N$ values of plants with mycorrhizal associations has been attributed to a fractionation of 8 to 10 $\%$ against $\delta^{15}N$ during the transfer of N from fungi to plants [162,163], with the lowest values indicating higher retention of N in the fungi compared to the plant [164].
Intraplant and temporal variation in plant $^{15}$N. There are three main reasons that plants exhibit intraplant and temporal variation in their tissue $^{15}$N values: (1) fractionations associated with NO$_3^-$ assimilation in the root vs. shoot, (2) movement of nitrogenous compounds between nitrogen sources and sinks, (3) reliance on isotopically variable N sources as tissue forms over time.

Both NO$_3^-$ and NH$_4^+$ are taken up by plant roots. NO$_3^-$ can be immediately assimilated into organic N in the root, or it may be routed to the shoot and assimilated there. The assimilation of NO$_3^-$ into organic N is associated with a fractionation of $^{15}$N of up to $-20\%$ [137,165]. Therefore, the NO$_3^-$ that is moved to

![Figure 3. Frequency distributions of carbon isotopic compositions of marine macroalgae. Data are taken from published literature [119,219–235].](image)

Table 1. Ecological zones used for sampling in this study [54].

| Zone                        | Altitude       |
|-----------------------------|----------------|
| Coastal desert              | 0 – 500 masl   |
| Premontane desert scrub     | 500 – 1,800 masl|
| Premontane thorny steppe    | 1,800 – 2,800 masl|
| Montane moist pasture       | 2,800 – 3,700 masl|
| Montane wet pasture         | 3,700 – 4,200 masl|

![Table 1. Ecological zones used for sampling in this study [54].](image)
Figure 4. Images of eight of the wild plant sampling locations. Corresponding geographical data for these sites can be found in Table 6.
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the shoot has already been exposed to some fractionation associated with assimilation and is enriched in $^{15}$N compared to the NO$_3^-$ that was assimilated in the root. On this basis, it is expected that shoots will have higher $^{15}$N values than roots in plants fed with NO$_3^-$ [166]. Because NH$_4^+$ is assimilated only in the root, plants with NH$_4^+$ as their primary N source are not expected to have significant root/shoot variation in $^{15}$N [136].

As plants grow they accumulate N in certain tissues (sources) and, over time, move this N to other tissues (sinks). In many species, annuals in particular, large portions of the plant’s resources are allocated to grain production or flowering. In these cases, significant portions of leaf and/or stem N is mobilized and allocated to the fruits, grains, or flowers [167]. When stored proteins are hydrolyzed, moved, and synthesized, isotopic fractionations occur [168,169]. Theoretically, nitrogen sources (leaves, stems) should be comparatively enriched in $^{15}$N in relation to sinks (grains, flowers), which has been observed in several studies [143,145,147].

Environmental factors affecting plant $^{15}$N. Plant nitrogen isotopic compositions are strongly influenced by a series of environmental factors. The environmental variation in plant $^{15}$N can be passed on to consumers and cause significant spatial variation in animal isotopic compositions at regional and continental scales [170–175].

Plant $^{15}$N values have been observed to be positively correlated with mean annual temperature (MAT) [176,177], although this relationship appears to be absent in areas where MAT $\leq -0.5^\circ$C [12]. A large number of studies have found a negative correlation between plant $^{15}$N values and local precipitation and/or water availability. These effects have been demonstrated at regional or continental [15,85–87,172,178], and global [12,176,179] scales. Several authors have hypothesized that relatively high $^{15}$N values in herbivore tissues may be the product of physiological processes within the animal related to drought stress [171,173,174], although controlled experiments have failed to provide any evidence supporting this hypothesis [180]. More recent research has demonstrated a clear link between herbivore tissue $^{15}$N values

| Site ID | Site Name       | Latitude | Longitude | Altitude (masl) |
|---------|-----------------|----------|-----------|-----------------|
| C1      | Caraz           | $-9.0554$| $-77.8101$| 2233            |
| C2      | Yungay          | $-9.1394$| $-77.7481$| 2468            |
| C3      | Jesus           | $-7.2448$| $-78.3797$| 2530            |
| C4      | Jesus II        | $-7.2474$| $-78.3821$| 2573            |
| C5      | Ampu            | $-9.2757$| $-77.6558$| 2613            |
| C6      | Shuto           | $-7.2568$| $-78.3807$| 2629            |
| C7      | Carhuaz         | $-9.2844$| $-77.6422$| 2685            |
| C8      | Yamobamba       | $-7.8432$| $-78.0956$| 3176            |
| C9      | Huamachucu      | $-7.7846$| $-77.9748$| 3196            |
| C10     | Curgos          | $-7.8599$| $-77.9475$| 3220            |
| C11     | Poc Poc         | $-7.9651$| $-77.8964$| 3355            |
| C12     | Recuay          | $-9.7225$| $-77.4531$| 3400            |
| C13     | Olleros         | $-9.6667$| $-77.4657$| 3437            |
| C14     | Hierba Buena    | $-7.0683$| $-78.5959$| 3453            |
| C15     | Mirador II      | $-9.7220$| $-77.4601$| 3466            |
| C16     | Yanac           | $-7.7704$| $-77.7979$| 3471            |
| C17     | Mirador I       | $-9.7224$| $-77.4601$| 3477            |
| C18     | Conray Chico    | $-9.6705$| $-77.4848$| 3530            |
| C19     | Catac           | $-9.8083$| $-77.4282$| 3588            |

Table 2. Environmental data for market plant sampling sites.

Figure 5. Carbon and nitrogen isotopic compositions of cultigens. Note that the x-axis is not continuous.

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and plant $^{15}$N values, while providing no support for the ‘physiological stress hypothesis’ [172,181].

The nature of the relationship between rainfall and plant $^{15}$N values appears to be extremely complex, with numerous variables contributing to the pattern. Several authors, including Handley et al. [179], have attributed this pattern to the relative ‘openness’ of the nitrogen cycle. In comparison to hot and dry systems, which are prone to losses of excess N, colder and wetter systems more efficiently conserve and recycle mineral N [176] and are thus considered less open. With respect to ecosystem $^{15}$N, $^{15}$N enrichment will be favored for any process that increases the flux of organic matter to mineral N, or decreases the flux of mineral N into organic matter [178]. For instance, low microbial activity, or high NH$_3$ volatilization would cause an overall enrichment in $^{15}$N of the soil-plant system.

**Marine plants.** In comparison to terrestrial plants, the factors affecting the nitrogen isotopic composition of marine plants have not been investigated intensively other than the influence of anthropogenic nitrogen. As is the case with terrestrial plants, marine plant $^{15}$N values are strongly influenced by the forms and isotopic composition of available N [182,183]. Specifically, the relative reliance on upwelled NO$_3^-$ relative to recycled NH$_4^+$ will strongly influence the $^{15}$N of marine producers, including macroalgae. Systems that are nutrient poor (oligotrophic) tend to be more dependent on recycled NH$_4^+$, and systems that are nutrient rich (eutrophic) tend to be more dependent on upwelled NO$_3^-$. This results in nutrient-rich, upwelling systems being enriched in $^{15}$N relative to oligotrophic systems [184].

**Materials and Methods**

**Sample Collection**

Wild plants were collected between 2011/07/18 and 2011/08/03. We used regional ecological classifications defined by Tosi [54], which are summarized in Table 1. In each of these five zones, two sites were selected that typified the composition of local vegetation. Sampling locations were chosen to minimize the possibility of significant anthropogenic inputs; in particular, areas close to agricultural fields and disturbed areas were avoided. Sampling locations were fairly open and did not have significant canopy cover. At each sampling location, all plant taxa within a 10 m radius were sampled. Wherever possible, three individuals of each species were sampled and were later homogenized into a single sample for isotopic analysis. Images for eight of the wild plant sampling locations are presented in Figure 4.

**Cultigens (edible portions) were collected from local markets between 2008/10/08 and 2008/11/09 (Table 2). Plants introduced to the Americas were not collected (e.g. peas, barley), even though these species were common.**

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### Table 3. Mean carbon and nitrogen isotopic compositions for cultigens ($\pm 1\sigma$).

| Common Name | Taxonomic Name       | $n$ | $\delta^{13}$C (% vs. VPDB) | $\delta^{15}$N (% vs. AIR) | %C | %N |
|-------------|----------------------|-----|-----------------------------|-----------------------------|----|----|
| Beans       | Phaseolus sp.        | 24  | $-25.7\pm1.6$               | $0.7\pm2.0$                 | 39.8$\pm0.7$ | 3.7$\pm0.6$ |
| Beans (Lima)| Phaseolus lunatus    | 2   | $-26.0\pm1.4$               | $-0.2\pm0.4$               | 39.0$\pm0.3$ | 2.7$\pm0.5$ |
| Chocho (Andean lupin) | Lupinus mutabilis      | 5   | $-26.0\pm1.6$               | $0.6\pm1.2$                 | 48.3$\pm2.8$ | 6.8$\pm1.3$ |
| Coca        | Erythroxylum coca     | 4   | $-29.8\pm0.9$               | --                          | 45.4$\pm1.5$ | -- |
| Maize (Grain)| Zea mays              | 27  | $-11.8\pm0.4$               | $6.4\pm2.2$                 | 40.4$\pm0.5$ | 1.2$\pm0.2$ |
| Maize (Leaf)| Zea mays              | 2   | $-12.9\pm0.4$               | $4.5\pm1.6$                 | 41.9$\pm4.6$ | 1.3$\pm1.3$ |
| Mashua      | Tropaeolum tuberosum  | 3   | $-25.6\pm1.9$               | $0.5\pm4.7$                 | 41.5$\pm2.8$ | 3.0$\pm0.7$ |
| Oca         | Oxalis tuberosa       | 6   | $-26.4\pm0.7$               | $5.7\pm1.3$                 | 43.1$\pm3.2$ | 1.6$\pm0.6$ |
| Pepper      | Capsicum annuum       | 1   | $-29.6$                     | $4.2$                       | 48.3 | 2.1 |
| Potato      | Solanum tuberosum     | 12  | $-26.3\pm1.3$               | $4.0\pm5.5$                 | 40.5$\pm1.5$ | 1.4$\pm0.4$ |
| Quinoa      | Chenopodium quinoa    | 3   | $-25.6\pm0.9$               | $7.9\pm1.3$                 | 39.9$\pm2.1$ | 2.6$\pm0.3$ |
| Ulluco      | Ullucus tuberosus     | 2   | $-25.8\pm0.0$               | $7.5\pm1.0$                 | 40.6$\pm0.4$ | 3.4$\pm1.0$ |

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### Table 4. Results of ANOVA post-hoc tests (Dunnett’s T3) for cultigen $^{15}$N.

| Cultigen  | %N | Bean (P. l. lunatus) | Andean lupin | Maize | Mashua | Oca | Potato | Quinoa | Ulluco |
|-----------|----|---------------------|-------------|-------|--------|-----|--------|--------|--------|
| Bean (P. l. lunatus) | 0.860 | 1.000 | <0.001 | 1.000 | 0.003 | 0.798 | 0.028 | 0.880 |
| Andean lupin | 0.971 | 0.001 | <0.001 | 1.000 | 0.005 | 0.479 | 0.037 | 0.121 |
| Maize      | 0.096 | 1.000 | 0.006 | 0.802 | 0.020 | 0.060 |
| Mashua     | 0.788 | 1.000 | 0.626 | 0.723 |
| Oca        | 0.688 | 0.780 |
| Potato     | 1.000 |
| Quinoa     | 1.000 |

Values in boldface are statistically significant ($p<0.05$).

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tubers) were selected and subsequently, a thin (ca. 0.5 cm) slice was sampled. For smaller cultigens (e.g. maize, beans, quinoa) one handful of material was sampled.

For both wild plants and cultigens, geospatial data were recorded using a Garmin® Oregon® 450 portable GPS unit (Garmin®, Olathe, KS, USA). After collection, plants were air-dried on site. Prior to shipping, plants were dried with a Salton® DH-1171 food dehydrator (Salton Canada, Dollard-des-Ormeaux, QC, Canada). Plants were separated according to tissue (leaf, stem, seed, flower). For grasses, all aboveground tissues were considered to be leaf except where significant stem development was present, in which case, leaf and stem were differentiated. All geospatial data associated with these sampling sites are available as a Google Earth.kmz file in the Supporting Information (Dataset S1).

Plants were not sampled from privately-held land or from protected areas. Endangered or protected species were not sampled. Plant materials were imported under permit #2011–03853 from the Canadian Food Inspection Agency. No additional specific permissions were required for these activities.

Sample Preparation

Samples were prepared according to Szpak et al. [143] with minor modifications. As described above, plant material was dried prior to arrival in the laboratory. Whole plant samples were first homogenized using a Magic Bullet® compact blender (Homeland Housewares, Los Angeles, CA, USA). Ground material was then sieved, with the <180 μm material retained for analysis in glass vials. If insufficient material was produced after sieving, the remaining material was further ground using a Wig-L-Bug.

![Figure 6. Dot-matrix plot of nitrogen isotopic compositions of legumes and non-legumes.](image.png)
mechanical shaker (Crescent, Lyons, IL, USA) and retained for analysis in glass vials. Glass vials containing the ground material were dried at 90°C for at least 48 h under normal atmosphere.

Stable Isotope Analysis

Isotopic ($\delta^{13}C$ and $\delta^{15}N$) and elemental compositions (%C and %N) were determined using a Delta V isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany) coupled to an elemental analyzer (Costech Analytical Technologies, Valencia, CA, USA), located in the Laboratory for Stable Isotope Science (LSIS) at the University of Western Ontario (London, ON, Canada). For samples with <2% N, nitrogen isotopic compositions were determined separately, with excess CO₂ being removed with a Carbo-Sorb trap (Elemental Microanalysis, Okehampton, Devon, UK) prior to isotopic analysis.

Sample $\delta^{15}C$ and $\delta^{15}N$ values were calibrated to VPDB and AIR, respectively, with USGS40 (accepted values: $\delta^{13}C = -26.39\%o$, $\delta^{15}N = -4.52\%o$) and USGS41 (accepted values: $\delta^{13}C = 37.63\%o$, $\delta^{15}N = 47.6\%o$). In addition to USGS40 and USGS41, internal (keratin) and international (IAEA-CH-6, IAEA-N-2) standard reference materials were analyzed to monitor analytical precision and accuracy. A $\delta^{13}C$ value of $-24.03\pm0.14\%o$ was obtained for 81 analyses of the internal keratin standard, which compared well with its average value of $-24.04\%o$. A $\delta^{13}C$ value of $-10.46\pm0.09\%o$ was obtained for 46 analyses of IAEA-CH-6, which compared well with its accepted value of $-10.45\%o$. Sample reproducibility was $\pm0.10\%o$ for $\delta^{13}C$ and $\pm0.50\%o$ for %C (50 replicates). A $\delta^{15}N$ value of $6.37\pm0.15\%o$ was obtained for 172 analyses of an internal keratin standard, which compared well with its average value of $6.36\%o$. A $\delta^{15}N$ value of $20.5\pm0.4\%o$ was obtained for 76 analyses of IAEA-N-2, which compared well with its accepted value of $20.3\%o$. Sample reproducibility was $\pm0.14\%o$ for $\delta^{15}N$ and $\pm0.10\%o$ for %N (84 replicates).

Data Treatment and Statistical Analyses

Plants were grouped into the following major functional categories for analysis: herb/shrub, tree, grass/sedge, vine. Plants

![Figure 7. Dot-matrix plot of nitrogen content of legumes and non-legumes. Horizontal bars represent means. Increment = 0.25%.](doi:10.1371/journal.pone.0053763.g007)
that are invasive and/or introduced species were included in the calculation of means for particular sites since their isotopic compositions should still be impacted by the same environmental factors as other plants. For all statistical analyses of carbon isotopic composition, grass/sedge and herb/shrub were further separated into C3 and C4 categories. For comparisons among plant functional types, and sampling sites, foliar tissue was used since other tissues were not as extensively sampled.

Correlations between foliar isotopic compositions and environmental parameters (altitude, mean annual precipitation) were assessed using Spearman’s rank correlation coefficient (ρ). One-way analysis of variance (ANOVA) followed by either a Tukey’s HSD test (if variance was homoscedastic) or a Dunnett’s T3 test (if variance was not homoscedastic) was used to compare means. All statistical and elemental regressions were performed in SPSS 16 for Windows.

Results

Cultigens

The carbon and nitrogen isotopic compositions were analyzed for a total of 85 cultigen samples from eleven species. Carbon and nitrogen isotopic compositions for cultigens are presented in Figure 5. Mean δ13C and δ15N values for cultigens are presented in Table 3. Isotopic and elemental data, as well as corresponding geospatial data for individual cultigens are presented in Table S1.

All isotopic and elemental compositions for cultigens are for consumable portions of the plant, with one exception (maize leaves), which is excluded from Table 3 and Figure 5. Mean δ13C values for C3 cultigens ranged from −29.8 ± 0.9 % (coca) to −25.6 ± 1.9 % (mashua). The mean δ13C value for maize, which was the only C4 plant examined, was −11.8 ± 0.4 % . Mean δ15N values for cultigens were typically more variable than δ13C values, ranging from −0.2 ± 0.4 % (Phaseolus lunatus) to 7.9 ± 1.3 % (quinoa).

When maize is excluded, there were no significant differences in δ13C among cultigens (F17,49 = 0.3, ρ = 0.93), but there were for δ15N (maize included) (F17,49 = 9.7, ρ < 0.001). Results of post-hoc Dunnett’s T3 test for δ15N differences among individual cultigen species are presented in Table 4. The three leguminous species are generally characterized by significantly lower δ15N values than non-leguminous species (Table 4); collectively, legumes were characterized by significantly lower δ15N values than non-legumes (Figure 6; F11,180 = 51.8, ρ < 0.001).

Table 6. Environmental data for wild plant sampling sites and summary of number of C3 and C4 plant species sampled.

| Site ID | Site Name     | Latitude | Longitude | Altitude (masl) | MAP (mm) 1 | C3 Plant Taxa Sampled | C4 Plant Taxa Sampled |
|---------|---------------|----------|-----------|-----------------|------------|------------------------|------------------------|
| W1      | Las Delicias  | −8.1956  | −78.9996  | 10              | 7          | 7                      | 2                      |
| W2      | Río Moche     | −8.1267  | −78.9963  | 33              | 5          | 9                      | 1                      |
| W3      | Ciudad Universitaria | −8.1137  | −79.0373  | 38              | 6          | 2                      | 0                      |
| W4      | Cerro Campana | −7.9900  | −79.0768  | 164             | 11         | 4                      | 1                      |
| W5      | La Carbonera  | −8.0791  | −78.8681  | 192             | 56         | 5                      | 3                      |
| W6      | Poroto        | −8.0137  | −78.7972  | 447             | 113        | 17                     | 6                      |
| W7      | Salpo 5       | −8.0089  | −78.6962  | 1181            | 143        | 0                      | 2                      |
| W8      | Salpo 4       | −8.0047  | −78.6726  | 1557            | 140        | 9                      | 0                      |
| W9      | Salpo 3       | −8.0132  | −78.6355  | 2150            | 141        | 16                     | 0                      |
| W10     | Salpo 2       | −7.9973  | −78.6481  | 2421            | 142        | 8                      | 0                      |
| W11     | Salpo 1       | −8.0132  | −78.6355  | 2947            | 171        | 9                      | 1                      |
| W12     | Stgo de Chucó | −8.1361  | −78.1685  | 3041            | 702        | 21                     | 1                      |
| W13     | Cahuide       | −8.2235  | −78.3013  | 4070            | 591        | 15                     | 0                      |

1 Mean annual precipitation (MAP) estimated as described in the text.

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Table 7. Carbon and nitrogen isotopic compositions for all wild plant taxa sampled.

| Taxonomic Name         | Site ID | Altitude | Type       | Leaf C (%) | Leaf N (%) | Stem C (%) | Stem N (%) | Root C (%) | Root N (%) | Flowers C (%) | Flowers N (%) | Seeds C (%) | Seeds N (%) |
|------------------------|---------|----------|------------|------------|------------|------------|------------|------------|------------|----------------|----------------|-------------|-------------|
| Eriochloa mutica       | W1      | 10       | Grass      | −11.6      | −1.5       | −11.7      | 1.6        | −          | −          | −27.0          | 4.1            | −26.9       | 4.2         |
| Distichia spicata      | W1      | 10       | Grass      | −14.9      | −3.2       | −          | −          | −          | −          | −27.0          | 4.1            | −26.9       | 4.2         |
| Baccharis glutinosa    | W1      | 10       | Shrub      | −27.4      | 3.3        | −26.6      | 5.1        | −          | −          | −28.7          | 2.6            | −           | −           |
| Rauvolfia sp.          | W1      | 10       | Shrub      | −28.0      | 9.8        | −27.9      | 11.0       | −          | −          | −              | −              | −           | −           |
| Plantago major¹        | W1      | 10       | Herb       | −28.6      | 7.5        | −27.5      | 7.9        | −26.7      | 8.6        | −              | −              | −           | −           |
| Typha angustifolia     | W1      | 10       | Herb       | −29.3      | 1.3        | −          | −          | −28.7      | 2.6        | −              | −              | −           | −           |
| Blumea crispa¹         | W1      | 10       | Herb       | −29.8      | 13.7       | −30.4      | 13.7       | −30.5      | 11.6       | −              | −              | −           | −           |
| Rosippa nastrutium aquaticum¹ | W1 | 10       | Herb       | −30.1      | 12.5       | −          | −          | −30.0      | 11.4       | −              | −              | −           | −           |
| Oxalis corniculata     | W1      | 10       | Herb       | −30.6      | 7.1        | −31.0      | 6.0        | −31.2      | 4.7        | −              | −              | −           | −           |
| Paspalum racemosum     | W2      | 33       | Grass      | −12.7      | 0.8        | −12.8      | 11.7       | −          | −          | −              | −              | −           | −           |
| Salix humboldtiana     | W2      | 33       | Tree       | −26.4      | 5.2        | −26.5      | 4.4        | −          | −          | −              | −              | −           | −           |
| Phyla nodiflora        | W2      | 33       | Herb       | −27.7      | 6.5        | −26.8      | 5.1        | −27.1      | 2.8        | −26.7          | 7.9            | −           | −           |
| Melochia lupulina      | W2      | 33       | Shrub      | −28.3      | 6.9        | −27.6      | 6.4        | −          | −          | −28.6          | 6.8            | −           | −           |
| Ipomoea alba           | W2      | 33       | Herb       | −28.7      | 9.3        | −28.1      | 8.1        | −          | −          | −              | −              | −27.3       | 8.9         |
| Persea americana       | W2      | 33       | Tree       | −28.8      | 1.9        | −26.8      | 7.0        | −          | −          | −              | −              | −           | −           |
| Ambrosia peruiana      | W2      | 33       | Herb       | −29.6      | 2.2        | −30.0      | 1.7        | −          | −          | −              | −              | −           | −           |
| Anundo donax¹          | W2      | 33       | Grass      | −30.3      | 8.5        | −30.1      | 10.2       | −          | −          | −              | −              | −           | −           |
| Acacia huansango²      | W2      | 33       | Shrub      | −31.0      | 3.5        | −30.0      | 2.3        | −          | −29.8      | 3.4            | −              | −           | −           |
| Pinnatanthus obovatus  | W2      | 33       | Shrub (Parasitic) | −31.9 | 5.1     | −30.6 | 6.2     | −          | −          | −29.1 | 5.8        | −              | −           | −           |
| Prosopis pallida²      | W3      | 38       | Tree       | −27.9      | 4.0        | −28.9      | 1.5        | −          | −29.1      | 5.8            | −              | −           | −           |
| Acacia macracantha²    | W3      | 38       | Tree       | −30.7      | 8.3        | −30.1      | 6.8        | −          | −30.5      | 8.6            | −28.9          | 5.1         |
| Tillandsia usneoides   | W4      | 164      | Epiphyte   | −13.6      | 3.7        | −14.2      | 1.9        | −13.9      | 14.5       | −13.6          | 0.0            | −           | −           |
| Cryptocarpus pyroformis| W4      | 164      | Shrub      | −22.5      | 10.3       | −22.2      | 10.4       | −          | −          | −              | −              | −22.4       | 12.1        |
| Triticis calacoiodes   | W4      | 164      | Shrub      | −26.6      | 9.2        | −26.0      | 7.6        | −          | −          | −              | −              | −25.7       | 9.4         |
| Scutia spicata         | W4      | 164      | Shrub      | −27.1      | 4.9        | −25.7      | 4.4        | −          | −          | −              | −              | −14.0       | 13.6        |
| Capparis angulata      | W4      | 164      | Shrub      | −27.3      | 10.0       | −27.7      | 10.7       | −          | −          | −26.0          | 11.6           | −           | −           |
| Paspalidium paladivagum| W5      | 192      | Grass      | −12.5      | 10.5       | −12.8      | 11.0       | −12.0      | 11.7       | −              | −              | −12.3       | 13.4        |
| Amaranthus celosiodes  | W5      | 192      | Herb       | −13.1      | 9.1        | −12.5      | 11.0       | −          | −13.5      | 10.9           | −12.2          | 8.6         |
| Tribulus terrestris    | W5      | 192      | Herb       | −15.6      | 11.8       | −16.2      | 14.4       | −          | −          | −              | −              | −14.0       | 13.6        |
| Hydrocotyle bonariensis| W5      | 192      | Herb       | −26.5      | 9.0        | −          | −          | −          | −          | −              | −              | −           | −           |
| Cestrum auriculatum    | W5      | 192      | Shrub      | −26.9      | 10.6       | −26.8      | 8.5        | −          | −          | −              | −              | −27.0       | 12.1        |
| Cucumis dipsaceus      | W5      | 192      | Herb       | −27.4      | 5.6        | −26.8      | 4.2        | −          | −          | −27.0          | 5.5            | −28.1       | 6.5         |
| Argemone subfuliformis | W5      | 192      | Herb       | −28.8      | 6.9        | −28.1      | 6.3        | −28.8      | 6.2        | −28.9          | 5.7            | −           | −           |
| Picrosia longifolia    | W5      | 192      | Herb       | −30.6      | 5.3        | −30.5      | 1.1        | −          | −          | −30.0          | 9.3            | −           | −           |
### Table 7. Cont.

| Taxonomic Name       | Site ID | Altitude | Type    | Leaf $\delta^{13}C$ ($\%$) | $\delta^{15}N$ ($\%$) | Stem $\delta^{13}C$ ($\%$) | $\delta^{15}N$ ($\%$) | Root $\delta^{13}C$ ($\%$) | $\delta^{15}N$ ($\%$) | Flowers $\delta^{13}C$ ($\%$) | $\delta^{15}N$ ($\%$) | Seeds $\delta^{13}C$ ($\%$) | $\delta^{15}N$ ($\%$) |
|----------------------|---------|----------|---------|-----------------------------|------------------------|---------------------------|------------------------|---------------------------|------------------------|---------------------------|------------------------|---------------------------|------------------------|
| Cylorhiza morphysa  | W6      | 447      | Sedge   | −13.1                       | 8.3                    | −11.2                      | 8.8                    | −11.7                      | 7.8                    | −14.2                      | 9.1                    | −                       | −                       |
| Echinocloa crusgalli | W6      | 447      | Grass   | −13.4                       | 2.8                    | −13.8                      | 2.8                    | −                         | −                      | −                         | −13.7                  | 3.9                      | −                       |
| Gynodiina dactylon  | W6      | 447      | Grass   | −13.9                       | 0.8                    | −                          | −                      | −                         | −                      | −14.1                      | 1.2                    | −                       | −                       |
| Sorghum halepense   | W6      | 447      | Grass   | −14.0                       | 2.5                    | −15.0                      | 4.7                    | −                         | −                      | −                         | −13.1                  | 3.7                      | −                       |
| Trianthema portulacastrum | W6    | 447     | Herb    | −14.2                       | 17.3                   | −13.7                      | 12.3                   | −                         | −                      | −                         | −                       | −                       | −                       |
| Amananthus spinusus | W6      | 447      | Herb    | −14.4                       | 13.3                   | −13.8                      | 16.1                   | −                         | −                      | −14.0                      | 15.3                   | −14.4                  | 15.3                    |
| Gymnestum sagittatum | W6      | 447      | Grass   | −25.8                       | 2.7                    | −25.1                      | 2.3                    | −                         | −                      | −25.6                      | 5.3                    | −                       | −                       |
| Alternanthera halimifolia | W6    | 447     | Herb    | −26.0                       | 8.4                    | −26.1                      | 9.2                    | −                         | −                      | −26.1                      | 8.2                    | −                       | −                       |
| Cissus sicyoides    | W6      | 447      | Vine    | −26.6                       | 10.9                   | −26.1                      | 12.4                   | −                         | −                      | −                         | −25.1                  | 11.9                    | −                       |
| Dalea onobrychis    | W6      | 447      | Herb    | −27.2                       | 8.7                    | −27.4                      | 7.8                    | −                         | −                      | −                         | −26.8                  | 7.4                      | −                       |
| Cleome spinosa      | W6      | 447      | Herb    | −27.3                       | 9.0                    | −27.1                      | 9.8                    | −                         | −                      | −                         | −26.8                  | 9.8                      | −                       |
| Crotalaria inca     | W6      | 447      | Shrub   | −27.3                       | 0.2                    | −26.6                      | 2.4                    | −                         | −                      | −25.4                      | 1.0                    | −26.0                  | −0.8                    |
| Ludwigia octovalvis | W6      | 447      | Herb    | −27.5                       | 0.6                    | −26.9                      | 1.3                    | −                         | −                      | −                         | −                       | −                       | −                       |
| Passiflora foetida  | W6      | 447      | Vine    | −27.5                       | 9.5                    | −27.2                      | 1.7                    | −                         | −                      | −                         | −27.5                  | 7.8                    | −                       |
| Wedelia fastiofolia | W6      | 447      | Shrub   | −28.0                       | 6.4                    | −27.3                      | 4.8                    | −                         | −                      | −26.4                      | 8.1                    | −                       | −                       |
| Baccharis salicifolia| W6      | 447      | Shrub   | −28.3                       | 6.5                    | −27.2                      | 8.4                    | −                         | −                      | −                         | −27.1                  | 8.0                      | −                       |
| Waltheria ovata     | W6      | 447      | Shrub   | −28.4                       | 6.1                    | −28.2                      | 5.9                    | −                         | −                      | −27.7                      | 6.0                    | −                       | −                       |
| Veibena littoralis  | W6      | 447      | Herb    | −28.8                       | 7.9                    | −28.4                      | 5.8                    | −                         | −                      | −27.7                      | 7.3                    | −                       | −                       |
| Cyporus odoratus    | W6      | 447      | Sedge   | −28.8                       | 9.2                    | −27.6                      | 10.1                   | −                         | −                      | −28.0                      | 10.2                   | −                       | −                       |
| Mimosa pigra       | W6      | 447      | Shrub   | −29.3                       | 1.7                    | −28.5                      | 0.3                    | −                         | −                      | −                         | −29.1                  | 1.3                      | −                       |
| Cajanus cajan 1,2  | W6      | 447      | Tree    | −29.6                       | 1.4                    | −28.4                      | −                      | −                         | −28.3                    | 0.3                      | −27.6                  | −0.7                    | −                       |
| Polygonum hydropiperoides | W6 | 447     | Herb    | −30.2                       | 6.8                    | −30.6                      | 6.7                    | −                         | −                      | −                         | −27.2                  | 8.1                      | −                       |
| Mimosa albida 2     | W6      | 447      | Shrub   | −30.5                       | 0.8                    | −30.1                      | 1.5                    | −                         | −                      | −                         | −28.8                  | 1.2                      | −                       |
| Melinis repens     | W7      | 1181     | Grass   | −13.3                       | 5.6                    | −13.4                      | 7.3                    | −                         | −                      | −                         | −14.5                  | 3.1                      | −                       |
| Chenmarus myosuroides | W7     | 1181     | Grass   | −13.3                       | 5.7                    | −                          | −                      | −                         | −                      | −                         | −                       | −                       | −                       |
| Dicliptera peruviana | W8    | 1557     | Herb    | −24.7                       | 3.9                    | −26.3                      | 3.2                    | −                         | −                      | −                         | −25.0                  | 3.2                      | −                       |
| Tournefortia micocalix | W8   | 1557     | Shrub   | −26.0                       | 6.4                    | −26.2                      | 6.0                    | −                         | −                      | −                         | −25.6                  | 7.2                      | −                       |
| Ophryosporus peruvianus | W8  | 1557     | Shrub   | −26.7                       | 2.9                    | −23.9                      | 1.8                    | −                         | −                      | −                         | −24.0                  | 2.6                      | −                       |
| Alternanthera porrigens | W8 | 1557     | Herb    | −27.8                       | 2.8                    | −27.2                      | 2.2                    | −                         | −                      | −                         | −25.7                  | 6.7                      | −                       |
| Asclepia curassavica | W8      | 1557     | Shrub   | −28.9                       | 2.6                    | −28.7                      | 4.2                    | −                         | −                      | −                         | −28.8                  | 0.4                      | −28.0                  | 0.2                    |
| Boerhavia erecta   | W8      | 1557     | Herb    | −29.3                       | 9.1                    | −28.3                      | 9.2                    | −                         | −                      | −                         | −                       | −                       | −                       |
| Centaura melitensis | W8      | 1557     | Herb    | −29.5                       | 0.5                    | −29.8                      | 0.2                    | −                         | −                      | −                         | −28.9                  | 1.7                      | −                       |
| Mentzella aspera    | W8      | 1557     | Herb    | −30.0                       | 1.0                    | −27.6                      | 6.8                    | −                         | −                      | −                         | −29.5                  | 1.3                      | −                       |
| Sida spinosa       | W8      | 1557     | Herb    | −30.1                       | 3.1                    | −30.1                      | 4.7                    | −                         | −                      | −                         | −31.3                  | 1.7                      | −                       |
| Taxonomic Name       | Site ID | Altitude | Type      | Leaf $\delta^{13}$C (‰) | Leaf $\delta^{15}$N (‰) | Stem $\delta^{13}$C (‰) | Stem $\delta^{15}$N (‰) | Root $\delta^{13}$C (‰) | Root $\delta^{15}$N (‰) | Flowers $\delta^{13}$C (‰) | Flowers $\delta^{15}$N (‰) | Seeds $\delta^{13}$C (‰) | Seeds $\delta^{15}$N (‰) |
|---------------------|--------|----------|-----------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Rubus robustus      | W9     | 2150     | Shrub     | -25.0                    | 3.0                      | -24.4                    | 2.7                      | -                        | -                        | -                        | -                        | -                        | -                        |
| Puya sp.            | W9     | 2150     | Succulent | -25.4                    | -0.7                     | -                        | -                        | -                        | -                        | -                        | -                        | -                        |
| Barnadesia dombeyana| W9     | 2150     | Shrub     | -26.0                    | -2.0                     | -25.4                    | -0.3                     | -                        | -                        | -25.7                    | -2.7                     | -                        | -                        |
| Iochroma edule      | W9     | 2150     | Shrub     | -26.1                    | 8.5                      | -25.8                    | 7.6                      | -                        | -                        | -                        | -                        | -25.4                    | 7.3                      |
| Eupatorium sp.      | W9     | 2150     | Herb      | -26.8                    | 2.5                      | -                        | -                        | -                        | -                        | -                        | -                        | -                        | -                        |
| Capparis scabrida   | W9     | 2150     | Shrub     | -26.8                    | 1.3                      | -26.3                    | 0.9                      | -                        | -                        | -26.5                    | 2.2                      | -                        | -                        |
| Vasquezia oppositifolia | W9     | 2150     | Herb      | -27.0                    | -1.6                     | -                        | -                        | -                        | -                        | -                        | -                        | -26.9                    | -1.3                     |
| Stipa ichu          | W9     | 2150     | Grass     | -27.0                    | 0.3                      | -                        | -27.4                    | 0.2                      | -27.3                    | 0.6                      | -                        | -                        |
| Lupinus sp.         | W9     | 2150     | Herb      | -27.1                    | 1.4                      | -27.1                    | 3.4                      | -                        | -26.6                    | 3.2                      | -26.7                    | 0.8                      |
| Alonsoa medionalis  | W9     | 2150     | Herb      | -27.5                    | 1.3                      | -26.4                    | -1.9                     | -                        | -                        | -                        | -25.9                    | 0.1                      |
| Bromus catharticus  | W9     | 2150     | Grass     | -27.8                    | 1.1                      | -29.3                    | -1.3                     | -                        | -                        | -                        | -27.5                    | -0.7                     |
| Baccharis sp.       | W9     | 2150     | Shrub     | -28.9                    | -1.1                     | -28.8                    | 0.1                      | -                        | -29.5                    | 0.5                      | -                        | -                        |
| Minthostachys mollis| W9     | 2150     | Herb      | -29.0                    | 0.5                      | -28.1                    | -1.6                     | -                        | -27.2                    | 0.1                      | -                        | -                        |
| Satureja sp.        | W9     | 2150     | Herb      | -30.2                    | -3.2                     | -                        | -                        | -29.8                    | -2.3                     | -                        | -                        |
| Achyrocline alata   | W9     | 2150     | Shrub     | -30.3                    | 0.3                      | -27.8                    | 1.2                      | -                        | -27.5                    | 2.0                      | -                        | -                        |
| Polygogon sp.       | W9     | 2150     | Grass     | -31.1                    | -5.3                     | -27.9                    | -4.4                     | -31.0                    | 2.4                      | -27.8                    | -3.7                     | -                        | -                        |
| Browalla americana  | W10    | 2421     | Herb      | -25.4                    | -1.6                     | -26.8                    | -2.5                     | -                        | -25.7                    | -0.8                     | -                        | -                        |
| Coniza sp.          | W10    | 2421     | Herb      | -26.7                    | 6.1                      | -26.1                    | 4.0                      | -                        | -                        | -                        | -                        | -                        |
| Heliotropium sp.    | W10    | 2421     | Herb      | -26.9                    | 3.7                      | -28.4                    | 2.2                      | -                        | -28.2                    | 3.2                      | -                        | -                        |
| Caesalpinia spinosa | W10    | 2421     | Tree      | -27.4                    | 2.7                      | -27.7                    | -0.4                     | -                        | -25.1                    | 0.0                      | -                        | -                        |
| Oenothera rosea     | W10    | 2421     | Herb      | -27.4                    | 4.9                      | -27.9                    | 4.6                      | -                        | -                        | -27.4                    | 2.9                      | -                        | -                        |
| Avena sterilis      | W10    | 2421     | Grass     | -27.5                    | 2.3                      | -27.2                    | 2.1                      | -27.0                    | 0.0                      | -                        | -22.5                    | 2.2                      | -                        | -                        |
| Berberis sp.        | W10    | 2421     | Shrub     | -27.7                    | 1.1                      | -24.6                    | 1.9                      | -                        | -26.7                    | 1.9                      | -                        | -                        |
| Alternanthera sp.   | W10    | 2421     | Herb      | -28.3                    | -2.9                     | -27.5                    | -3.0                     | -                        | -27.2                    | -0.8                     | -                        | -                        |
| Pennisetum purpureum| W11    | 2947     | Grass     | -12.5                    | 7.2                      | -12.8                    | 6.6                      | -                        | -                        | -15.5                    | 7.1                      | -                        | -                        |
| Ruella floribunda   | W11    | 2947     | Herb      | -23.7                    | 4.5                      | -24.0                    | 1.9                      | -                        | -23.5                    | 4.9                      | -                        | -                        |
| Schinus mollis      | W11    | 2947     | Tree      | -24.6                    | 2.3                      | -23.4                    | 0.3                      | -                        | -21.3                    | 0.8                      | -                        | -                        |
| Spartium junceum    | W11    | 2947     | Shrub     | -26.5                    | 1.1                      | -27.1                    | -1.1                     | -                        | -23.7                    | -1.3                     | -25.4                    | 0.8                      |
| Acacia aroma        | W11    | 2947     | Tree      | -26.8                    | 9.6                      | -26.6                    | 9.6                      | -                        | -26.6                    | 10.1                     | -                        | -                        |
| Croton ovalifolius  | W11    | 2947     | Shrub     | -27.0                    | 7.4                      | -27.6                    | 5.8                      | -                        | -                        | -27.1                    | 1.1                      | -                        | -                        |
| Leonotis nepetifolia| W11    | 2947     | Shrub     | -28.0                    | 2.2                      | -                        | -                        | -27.2                    | 3.0                      | -26.1                    | 2.0                      | -                        | -                        |
| Lycianthes lycoides | W11    | 2947     | Shrub     | -28.0                    | -0.3                     | -                        | -                        | -                        | -                        | -24.3                    | 2.0                      | -                        | -                        |
| Phexan hirtus       | W11    | 2947     | Shrub     | -28.3                    | 2.5                      | -29.1                    | 7.1                      | -                        | -                        | -28.5                    | 6.9                      | -                        | -                        |
| Inga feuillea       | W11    | 2947     | Tree      | -28.9                    | 0.3                      | -27.6                    | -0.8                     | -                        | -                        | -27.1                    | 1.1                      | -                        | -                        |
### Table 7. Cont.

| Taxonomic Name              | Site ID | Altitude | Type   | Leaf | Stem | Root | Flowers | Seeds |
|-----------------------------|---------|----------|--------|------|------|------|---------|-------|
| Andropogon sp.              | W12     | 3041     | Grass  | -13.5| -1.6 | -12.2| -10.2   | -     |
| Sebastiania obtusifolia     | W12     | 3041     | Shrub  | -23.7| 0.8  | -24.7| 0.0     | -240  |
| Lupinus aridus              | W12     | 3041     | Herb   | -24.3| 2.0  | -23.7| 2.2     | -227  |
| Silphium marianum           | W12     | 3041     | Herb   | -25.9| 2.2  | -25.8| 1.6     | -251  |
| Physilanthus sp.            | W12     | 3041     | Shrub (Parasitic) | -25.9| -0.5 | -24.7| 7.3     | -     |
| Solanum amoretapense        | W12     | 3041     | Shrub  | -25.9| 7.9  | -25.2| 5.1     | -247  |
| Acacia sp.                  | W12     | 3041     | Tree   | -26.2| -1.0 | -25.0| -2.5    | -     |
| Baccharis serpifolia        | W12     | 3041     | Shrub  | -26.4| 2.2  | -27.1| 1.5     | -26.9 |
| Aristida adsessionis        | W12     | 3041     | Grass  | -26.5| -2.6 | -26.2| -2.0    | -26.7 |
| Baccharis emarginata        | W12     | 3041     | Shrub  | -26.5| -0.2 | -25.4| 0.1     | -     |
| Brassica campestris         | W12     | 3041     | Herb   | -27.1| 2.3  | -27.6| 0.8     | -25.4 |
| Mauila sp.                  | W12     | 3041     | Tree   | -27.2| 6.1  | -25.8| 3.1     | -     |
| Solanum agronomiaeolium     | W12     | 3041     | Herb   | -28.0| 6.0  | -28.2| 3.8     | -266  |
| Salvia punctata             | W12     | 3041     | Herb   | -28.1| -3.5 | -27.6| -2.7    | -27.7 |
| Duranta sp.                 | W12     | 3041     | Shrub  | -28.4| 1.3  | -27.6| 0.8     | -     |
| Flourensia cajambensis      | W12     | 3041     | Shrub  | -28.6| 2.9  | -27.5| 2.7     | -29.4 |
| Marnubium vulgare           | W12     | 3041     | Herb   | -28.8| 3.8  | -26.6| 1.4     | -26.9 |
| Scutelaria sp.              | W12     | 3041     | Herb   | -28.8| 2.8  | -28.6| 0.6     | -     |
| Viguiera peruviana          | W12     | 3041     | Shrub  | -28.9| 5.3  | -27.3| 4.6     | -266  |
| Jungia rugosa               | W12     | 3041     | Shrub  | -28.9| 1.4  | -26.8| 1.1     | -27.1 |
| Saccellium sp.              | W12     | 3041     | Shrub  | -29.0| 2.0  | -27.3| 1.0     | -     |
| Baccharis libertadensis      | W12     | 3041     | Shrub  | -29.6| 3.8  | -28.4| 1.9     | -     |
| Ursnea andina               | W13     | 4070     | Lichen | -30.5| -6.5 | -     | -       | -     |
| Astragalus garbancillo      | W13     | 4070     | Shrub  | -24.6| 4.2  | -25.3| 3.0     | -23.8 |
| Luzula sp.                  | W13     | 4070     | Sedge  | -25.1| 0.9  | -25.0| 3.9     | -25.1 |
| Distichia muscoides         | W13     | 4070     | Grass  | -25.3| 4.4  | -25.2| 2.9     | -     |
| Muehlenbeckia sp.           | W13     | 4070     | Herb   | -25.3| 6.3  | -25.7| 4.9     | -     |
| Urtica sp.                  | W13     | 4070     | Shrub  | -25.5| 11.9 | -25.1| 9.0     | -26.0 |
| Agrostis breviculmis        | W13     | 4070     | Grass  | -25.9| 2.1  | -26.0| 4.1     | -25.5 |
| Chuiquira sp.               | W13     | 4070     | Shrub  | -26.0| -0.5 | -24.9| -1.1    | -24.3 |
| Weneria nubigena            | W13     | 4070     | Herb   | -26.2| 1.3  | -25.8| 1.8     | -     |
| Festuca dolichopyla         | W13     | 4070     | Grass  | -26.3| -1.8 | -25.4| -0.3    | -26.5 |
| Hypochaeris sp.             | W13     | 4070     | Herb   | -26.6| 7.3  | -26.9| 8.2     | -     |
| Plantago tubulosa           | W13     | 4070     | Herb   | -26.9| -5.2 | -26.0| -3.0    | -     |
Cultigen N content is presented in Table 3 and Figure 7. Mean %N for cultigens ranged from 1.2±0.2% (maize) to 6.8±1.3% (Andean lupin). Results of post-hoc Dunnett’s T3 test for differences between individual cultivar species in N content are presented in Table 5. The three leguminous species were characterized by significantly higher N contents than non-leguminous species (Table 5); collectively, legumes were characterized by significantly higher %N values than non-legumes (Figure 7; F_{[1,103]} = 116.0, p<0.001).

**Wild Plants**

A total of 139 species were sampled primarily from ten sites distributed along an altitudinal transect from 10 to 4,070 masl. The number of taxa sampled and environmental variables for each of the sampling locations are presented in Table 6. The number of C4 plant taxa was generally higher at lower altitude sites receiving lower amounts of rainfall. This fits with what is known about the global distribution of C4 plants [185].

The carbon and nitrogen isotopic compositions were measured for all 139 species. Foliar tissue was analyzed from all species, and additional tissues analyzed included: 112 stems, 28 roots, 51 flowers, and 62 seeds. Carbon and nitrogen isotopic compositions for wild plants are presented in Table 7 according to plant part. Foliar δ15N values for C3 plants ranged from −31.9 to −22.2 ‰, with a mean value of −27.6±1.9 ‰ (n = 122). Foliar δ13C values for C3 plants ranged from −15.6 to −11.6 ‰, with a mean value of −15.5±1.0 ‰ (n = 17). Foliar δ15N values for C4 plants ranged from −4.1 to 13.0 ‰, with a mean value of 3.7±4.0 ‰. Foliar δ15N values for C4 plants ranged from −3.2 to 15.0 ‰, with a mean value of 5.5±5.7 ‰. The single lichen analyzed (Usnea andina) was characterized by a δ13C value intermediate between C3 and C4 plants (−20.5 ‰) and a very low δ15N value (−6.5 ‰), consistent with previously reported results for lichens [71–73].

There were no significant differences in foliar δ15N among plant functional groups (F_{[3,132]} = 1.8, p = 0.15). Foliar δ13C differed significantly among plant functional groups (F_{[3,130]} = 195.0, p<0.001), although this was driven by differences between C3 and C4 groups; there were no significant differences in foliar δ15N between plant functional groups within C3 and C4 groups (Table 8).

There was no clear pattern of intraplant variation in δ15N (Figure 8) with differences in δ15N between tissues (Δ15N) being highly variable: Δ15Nstem–leaf = −0.3±2.3 ‰, Δ15Nroot–leaf = 0.4±3.1 ‰, Δ15Nflower–leaf = 0.5±1.4 ‰, Δ15Nseed–leaf = 0.5±1.7 ‰. Conversely, foliar tissue was typically characterized by lower δ15N values than all other tissues analyzed (Figure 9), and intraplant variation was generally smaller: Δ15Cstem–leaf = 0.5±0.9 ‰, Δ15Croot–leaf = 0.4±0.8 ‰, Δ15Cflower–leaf = 0.6±1.0 ‰, Δ15Cseed–leaf = 0.5±1.7 ‰. For C4 plants (n = 17), there was no clear pattern of intraplant variation in δ13C: Δ13Cstem–leaf = 0.0±0.8 ‰, Δ13Croot–leaf = 0.5±0.7 ‰, Δ13Cflower–leaf = −0.3±0.6 ‰, Δ13Cseed–leaf = −0.2±1.3 ‰.

Foliar nitrogen isotopic compositions for wild legumes (Fabaceae) were highly variable, ranging from −1.4 to 9.6 ‰. Among Acacia trees and shrubs alone, foliar δ15N values ranged from −1.0 to 9.6 ‰, suggesting that some species are not engaged in active N2 fixation. While wild legumes were characterized by lower foliar δ15N values relative to non-legumes (4.1±4.4 ‰, n = 119 for non-legumes; 2.7±3.4 ‰, n = 17 for legumes), this difference was not statistically significant (F_{[1,134]} = 1.8, p = 0.18).

Mean wild C4 plant foliar δ13C and δ15N values for sampling locations with ≥5 species sampled are presented in Table 9. Mean foliar carbon and nitrogen isotopic compositions for these sites are plotted against altitude in Figure 10 and estimated mean annual precipitation in Figure 11. Mean foliar δ15N values at low altitude
sites were 2 to 8 % higher than mean foliar $\delta^{15}$N values at high altitude sites. Foliar $\delta^{13}$N was negatively correlated with mean annual precipitation (Spearman’s $r = -0.770$, $p = 0.009$) and altitude (Spearman’s $r = -0.782$, $p = 0.008$). Foliar $\delta^{13}$C was positively correlated with mean annual precipitation (Spearman’s $r = 0.879$, $p = 0.001$) and altitude (Spearman’s $r = 0.903$, $p < 0.001$). For comparative purposes, mean plant $\delta^{13}$C values for sites sampled along an altitudinal transect in northern Chile are presented in Figure 12 [14].

**Marine Plants**

The carbon and nitrogen isotopic compositions were determined for a total of 25 marine plant samples from five species. Mean $\delta^{13}$C and $\delta^{15}$N values for marine plants are presented in Table 10. Mean $\delta^{13}$C values for marine plants ranged from $-18.7 \pm 0.7$ % (Gymnogongrus furcellatus) to $-14.2 \pm 1.2$ % (Grateloupia doryphora). Mean $\delta^{15}$N values for marine plants ranged from $2.5 \pm 0.9$ % (Gymnogongrus furcellatus) to $7.8 \pm 0.1$ % (Cryptopleura cryptoneuron). Overall, marine plants were characterized by $\delta^{13}$C values that were intermediate between C$_3$ and C$_4$ plant isotopic compositions, although more similar to the latter. In comparison to wild plants growing at the three sites located closest to the coast, marine plants were not characterized by significantly higher $\delta^{15}$N values when the plants from the three terrestrial sites are treated separately ($F[3,39] = 0.5$, $p = 0.71$) or grouped together ($F[1,41] < 0.1$, $p = 0.91$).

**Discussion**

**Cultigens**

The carbon isotopic composition of maize was $\sim 2$ % more enriched in $^{13}$C than wild C$_4$ plants (all tissues), similar to previously determined values for other parts of the world [186,187]. This suggests that a $\delta^{13}$C value of $-10.3$ % (adjusted by $+1.5$ % for the Suess Effect [188,189]) would be appropriate for

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**Table 8. Results of ANOVA post-hoc tests (Dunnett’s T3) for foliar $\delta^{13}$C between plant functional groups.**

| Foliar $\delta^{13}$C | C$_4$ Grass/Sedge | C$_4$ Herb/Shrub | C$_3$ Herb/Shrub | Tree | Vine |
|----------------------|-------------------|------------------|------------------|------|------|
| C$_4$ Grass/Sedge    | <0.001            | 0.999            | <0.001           | <0.001 | <0.001 |
| C$_4$ Grass/Sedge    | -                 | 0.993            | 1.000            | 1.000 |
| C$_4$ Herb/Shrub     | -                 | -                | 0.999            | <0.001 | <0.001 |
| C$_3$ Herb/Shrub     | -                 | -                | -                | 0.997 | 0.994 |
| Tree                 | -                 | -                | -                | -    | 1.000 |

Values in boldface are statistically significant ($p < 0.05$).

**Figure 8. Dot-matrix plot of differences in nitrogen isotopic composition between foliar and other tissues ($\Delta^{15}$N).** Horizontal bars represent means. Increment $= 0.5$ %.

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paleodietary models in the central Andes. There may, however, be some small-scale environmental effects on maize $\delta^{13}C$ values along an altitudinal gradient as discussed in more detail below.

For the most part, the $\delta^{15}N$ values of the modern cultigens presented in this study should be interpreted cautiously with respect to paleodietary studies. The primary factor influencing the nitrogen isotopic composition of plant tissues is the N source, and it cannot be assumed that modern N sources are directly analogous to those used in antiquity. The nitrogen isotopic composition of locally grown produce sold in Andean markets today may be influenced by chemical fertilizers (which cause plants to have relatively low nitrogen isotopic compositions) or by animal manures (e.g. sheep, cow, pig) that would not have been available in the region prior to the arrival of the Spanish. The same is true for nitrogen isotopic data obtained from modern agricultural plants globally, and as a general rule, the limitations of these data must be recognized. Nevertheless, some patterns are likely to be broadly applicable.

In contrast to the vast majority of published literature [27,138–140,190–200], Warinner et al. [187] showed very little distinction between the nitrogen isotopic composition of Mesoamerican legumes and non-legumes, suggesting that the assumption of lower $\delta^{15}N$ values in legumes in that region is tenuous. Where the potential effects of nitrogenous fertilizers on legume $\delta^{15}N$ values are unknown (as is the case for the data presented by Warinner et al. [187]), the interpretation of $\delta^{15}N$ values in legumes and

Figure 9. Dot-matrix plot of differences in carbon isotopic composition between foliar and other tissues ($\Delta^{13}C$). Horizontal bars represent means. Increment = 0.5‰.

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| Site ID | Latitude | Longitude | Altitude (masl) | MAP (mm) | $n^2$ | $\delta^{13}C$ (%/VPDB) | $\delta^{15}N$ (%/AIR) |
|---------|----------|-----------|----------------|----------|------|-----------------------|-----------------------|
| W1      | -8.1956  | -78.9996  | 10             | 7        | 7    | -29.1 ± 1.2           | 7.9 ± 4.5             |
| W2      | -8.1267  | -78.9696  | 33             | 5        | 9    | -29.2 ± 1.7           | 5.5 ± 2.6             |
| W5      | -8.0791  | -78.8681  | 192            | 56       | 5    | -28.1 ± 1.7           | 7.5 ± 2.2             |
| W6      | -8.0137  | -78.7972  | 447            | 113      | 17   | -28.1 ± 1.4           | 5.4 ± 4.0             |
| W8      | -8.0047  | -78.6726  | 1557           | 140      | 9    | -28.1 ± 1.9           | 3.6 ± 2.7             |
| W9      | -8.0132  | -78.6355  | 2150           | 141      | 16   | -27.6 ± 1.8           | 0.4 ± 3.0             |
| W10     | -7.9973  | -78.6481  | 2421           | 142      | 8    | -27.2 ± 0.8           | 2.0 ± 3.1             |
| W11     | -8.0132  | -78.6355  | 2947           | 171      | 9    | -26.9 ± 1.8           | 3.3 ± 3.3             |
| W12     | -8.1361  | -78.1685  | 3041           | 702      | 21   | -27.3 ± 1.6           | 2.1 ± 2.8             |
| W13     | -8.2235  | -78.3013  | 4070           | 591      | 15   | -26.0 ± 2.0           | 2.0 ± 4.9             |

1. Mean annual precipitation (MAP) estimated as described in the text.
2. Number of C$_3$ plant species sampled.

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non-legumes is not straightforward. While there was some overlap in δ¹⁵N values between legumes and non-legumes in this study, leguminous cultivars had significantly higher N contents (Figure 7; Table 5) and significantly lower δ¹⁵N values (Figure 6; Table 4) than non-legumes.

Aside from the differences in δ¹⁵N between legumes and non-legumes, it is very difficult to generalize the δ¹⁵N values for
cultigens in this study. Nitrogen isotopic compositions were highly variable, particularly for potato, which most likely reflected variable local growing conditions (soil fertility, type of manure used) rather than any biochemical or physiological process specific to any particular plant species. Ultimately, the best source of baseline isotopic data for paleodietary studies may be from archaeobotanical remains [27,201–203], provided that preservation of original carbon and nitrogen isotopic compositions can be demonstrated. Considerable work has been done in this regard for the isotopic composition of bone collagen [204–208] and to a lesser extent hair keratin [209], but a solid set of parameters for detecting preservation versus alteration of original plant carbon and nitrogen isotopic compositions have not yet been determined. The excellent organic preservation at many archaeological sites on the coasts of Peru and Chile provides the potential for such analyses to be conducted on botanical remains.

Figure 11. Bivariate plots of foliar δ¹⁵N and mean annual precipitation (A) and foliar δ¹³C (B) for C₃ plants only. Points represent means ±1σ for sites with ≥5 C₃ plant species sampled. Equation for δ¹⁵N and MAP: y = 8.8–1.1 logx, r² = 0.49; p = 0.03. Equation for δ¹³C and MAP: y = −30.1+0.5 logx, r² = 0.81; p < 0.001.
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Wild Plants

**Plant Functional Group.** There were no clear distinctions between different plant functional groups (grass, herb, shrub, tree, vine) with respect to either carbon or nitrogen isotopic compositions. While some systematic variation may be expected due to variable nitrogen acquisition strategies (e.g. rooting depth) or differential distribution of biomolecules with distinct isotopic compositions, the diverse range of environmental conditions from which plants were sampled likely served to blur any isotopic distinctions between functional groups. Moreover, the sample sizes for different plant functional groups within any one site were too small for meaningful comparisons to be made.

There was no consistent pattern in plant δ^{15}N with respect to leguminous trees and shrubs, with some species having foliar δ^{15}N values close to 0 %, and others having relatively high δ^{15}N values. Previous studies have similarly found conflicting patterns of relatively high and low δ^{15}N values in leguminous trees. Codron et al. [13] found no clear distinction between leguminous and non-leguminous trees at a regional scale in South Africa. Aranibar et al. [178] did not observe significant amounts of N₂-fixation among leguminous trees in an arid region of southern Africa, with trees growing at the most arid sites showing no evidence of N₂-fixation. Fruit-bearing trees of the genus *Prosopis* (often called huarango or algarrobo) are suggested to have been an important food source for various groups in the Andean region [64,210]. Catenazzi and Donnelly [28] found δ^{15}N values typical of N₂-fixing trees (ca. 0 %) in *Prosopis pallida* from the Sechura Desert of northern Peru. Conversely, on the basis of the isotopic data recorded in this study for leguminous trees in the Moche River Valley, the assumption that *Prosopis* would be characterized by significantly lower δ^{15}N values relative to other plants is tenuous. Given the potential importance of these foods in the diet, a more extensive study of the nitrogen isotopic composition of central Andean leguminous trees would be beneficial.

**Intraplant Variation in Carbon and Nitrogen Isotopic Compositions.** Plant nitrogen isotopic composition did not systematically vary between different tissues sampled. On the basis of hydroponic studies, significant intraplant variation (between roots and shoots) is only expected when plants are fed with NO₃⁻ as the N source [166]. Additionally, plant δ^{15}N may vary considerably among tissues due to biochemical processes associated with growth and senescence over time [143,211–213]. The lack of any clear pattern of intraplant variation in δ^{15}N likely relates to a number of factors, including: variable reliance on different N sources (nitrate, ammonium, organic N) by different plant taxa and between sampling locations, differences in plant life

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**Table 10.** Mean (±1σ) isotopic and elemental compositions for marine algae.

| Taxonomic Name       | Type     | n  | δ^{13}C (% vs. VPDB) | δ^{15}N (% vs. AIR) | %C  | %N  |
|----------------------|----------|----|---------------------|---------------------|-----|-----|
| *Ulva lactuca*       | Chlorophyta | 5  | -14.3±0.4           | 6.4±0.1             | 29.2±0.4 | 3.6±0.2 |
| *Gymnogongrus furcellatus* | Rhodophyta | 5  | -18.7±0.7           | 2.5±0.9             | 23.3±2.4 | 2.1±0.2 |
| *Grateloupia doryphora* | Rhodophyta | 5  | -14.2±1.2           | 6.8±0.3             | 29.9±1.0 | 3.2±0.1 |
| *Gigartina chamissai* | Rhodophyta | 5  | -16.7±1.0           | 5.4±0.5             | 25.7±0.4 | 2.7±0.1 |
| *Cryptopleura cryptoneuron* | Rhodophyta | 5  | -18.4±0.4           | 7.8±0.1             | 21.2±1.6 | 2.8±0.4 |

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Figure 12. Bivariate plot of altitude and foliar δ^{13}C for plants collected in northern Chile [14].
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cycles between different taxa, and spatial variation in the influence of environmental factors on the isotopic composition of source N.

Foliar tissues tended to be more depleted of $^{13}$C than other tissues (Figure 9). The magnitude of this difference was typically $\leq 1 \%$, but was absent for C$_4$ plants. This fits with previously described data for other plants. The small difference in $^{13}$C among plant tissues is not likely to be significant with respect to the interpretation of isotopic data in the context of paleodiets.

**Geographic Variation in Carbon and Nitrogen Isotopic Compositions.** There were strong relationships between sampling site and foliar carbon and nitrogen isotopic compositions. Foliar $^{13}$N was negatively correlated with altitude (Figure 10a) and mean annual precipitation (Figure 11a), although based on the large number of studies finding a strong relationship between rainfall amount and soil, plant, and animal $^{15}$N values [12,15,85–87,172,176–179], this relationship is likely driven by rainfall. This suggests that arid sites are characterized by a fairly open nitrogen cycle, as described in previous studies [179]. It is unclear to what extent these processes would act on agricultural plants growing in relatively arid versus wet sites. Even on the hyper-arid coast where rainfall is negligible, agriculture is made possible by substantial irrigation networks. Hence, water availability in agricultural contexts is markedly higher than in non-irrigated areas. Agricultural products grown in coastal regions of the central Andes may therefore not be characterized by higher $^{15}$N values relative to those growing at wetter, higher altitude sites. For instance, maize grown as part of a controlled experiment (no fertilization) located ~6 km from the coast, had high $^{15}$N values of 6.3±0.3 % (147), comparable to results for maize growing at higher altitudes in this study (6.4±2.2 %). Aside from issues of irrigation, agricultural plants analyzed in this study were sampled along a relatively limited altitudinal transect (2233 to 3588 masl) where effects on tissue $^{15}$N values would be expected to be more limited (Figure 10a).

The positive relationship found between rainfall and foliar $^{13}$C in C$_3$ plants contrasts with most other studies, which have typically found a negative relationship between rainfall and foliar $^{13}$C. The majority of these studies, however, sampled plants along a large rainfall gradient (>1,000 mm), but with little difference in elevation between sites. Conversely, we sampled along a more restricted rainfall gradient (~700 mm), but a very large altitudinal gradient (~4,000 m). Increased altitude and increased rainfall have opposing effects on foliar $^{13}$C values, and the results of this study suggest the predominance of altitudinal effects on foliar carbon isotopic compositions in northern Peru. A similar pattern was observed along a comparable altitudinal gradient in northern Chile (Figure 12). This pattern is most likely related to the complex climatic factors acting on plant physiology at higher altitude sites, with lower $^{13}$C discrimination. Such effects should be equally apparent in cultivated plants, although they were not observed in this study because of the limited altitudinal range from which cultivars were sampled (Table 2).

Variation in plant isotopic compositions along environmental gradients is particularly important with respect to the reconstruction of the diet of humans and animals using isotopic data. While the majority of wild plants analyzed in this study would not have been consumed by humans, the results are relevant to the reconstruction of animal management practices. There is considerable debate in the Andean region with respect to the herding practices of South American camels [llama and alpaca], and whether or not animals recovered from coastal sites were raised locally, or imported from elsewhere [214]. The results of this study suggest that animals feeding on wild plants at drier, low altitude sites would be characterized by higher tissue $^{15}$N values than animals feeding on wild plants at wetter, high altitude sites. The magnitude of this difference could easily be 4 to 6 %, although the consumption of agricultural plants dependent on irrigation at lower altitudes could serve to obscure this difference (as discussed above).

The potential consequences of altitudinal variation in plant $^{13}$C values are more difficult to evaluate. While the positive linear relationship between altitude and foliar $^{13}$C is strong, the relative distribution of C$_3$ and C$_4$ plants would serve to counter these effects. Because there will be proportionately more C$_4$ plants at dry, low altitude sites relative to moister, high altitude sites, the average $^{13}$C value of available forage would still be higher at low altitude sites. Thus, markedly higher $^{13}$C and $^{15}$N values observed in some camelids from low altitude sites [38,215] can be satisfactorily explained by the consumption of local terrestrial vegetation.

**Marine Plants**

Marine algae are known to have been an important dietary resource for many groups of people in the coastal regions of Peru and Chile [216], but the lack of preservation of marine algae in all but the most exceptional archaeological contexts makes evaluating the potential importance of marine algae in the diet extremely difficult. Marine plants were characterized by $^{13}$C values intermediate between C$_3$ and C$_4$ plants, with $^{15}$N values comparable to terrestrial plants growing on the coast. DeNiro [215] has suggested that consumption of marine algae may have been responsible for relatively high $^{13}$C and $^{15}$N values in coastal Peruvian camelids. While the number of macroalgal species sampled in this study is not extensive, the isotopic data presented here are not consistent with this explanation. With the exception of instances in which marine plants grow in areas of exceptionally high influence of marine birds and/or mammalian excreta [217], there is no reason to expect marine algal $^{15}$N values to be higher than the $^{15}$N values of plants growing along the arid coast of Peru.

**Conclusions**

Maize from the study area has a mean $^{13}$C value of $-11.8\pm0.4 \%$, which suggests that a $^{13}$C value (adjusted for the Suess Effect) of ca. $-10.3 \%$ would be appropriate for paleodietary models in the region. Leguminous cultigens were characterized by significantly lower $^{13}$N values and higher N contents than non-leguminous cultigens; this distinction was not as clear for wild legumes. Marine plants were characterized by $^{13}$C values intermediate between wild terrestrial C$_3$ and C$_4$ vegetation and $^{15}$N values that were very similar to terrestrial plants growing at low altitudes. C$_4$ plants were generally more abundant at higher altitude sites. Carbon and nitrogen isotopic compositions of wild plants were strongly influenced by local environmental factors. Foliar $^{13}$C was positively correlated with altitude and negatively correlated with mean annual precipitation. Foliar $^{15}$N was negatively correlated with altitude and mean annual precipitation.

While the last twenty years have seen a proliferation of studies utilizing the isotopic analysis of archaeological materials for the purpose of reconstructing diet, the development of isotopic baselines for interpreting such data has lagged behind these investigations. This hampers our ability to realize the full potential of isotopic data. This study begins to fill part of that gap by providing an initial understanding of the baseline isotopic variation in plants from northern Peru. Further studies of this nature are required to better understand baseline isotopic variation in other regions.
Supporting Information

Dataset S1 Sampling site locations for wild and market plants. This.kmz file can be executed in Google Earth (http://www.earth.google.com).

Table S1 Isotopic and elemental data for all cultigens analyzed. (XLS)

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