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 (δ^{13}C) and nitrogen (δ^{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 δ^{13}C value for maize (grain) was -11.8 ± 0.4 ‰ ($n=27$). Leguminous cultigens (beans, Andean lupin) were characterized by significantly lower δ^{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 δ^{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 C$_3$ plants, foliar δ^{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 δ^{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 δ^{13}C values for plants along a more limited altitudinal transect in Chile (Atacama Desert). A number of other studies
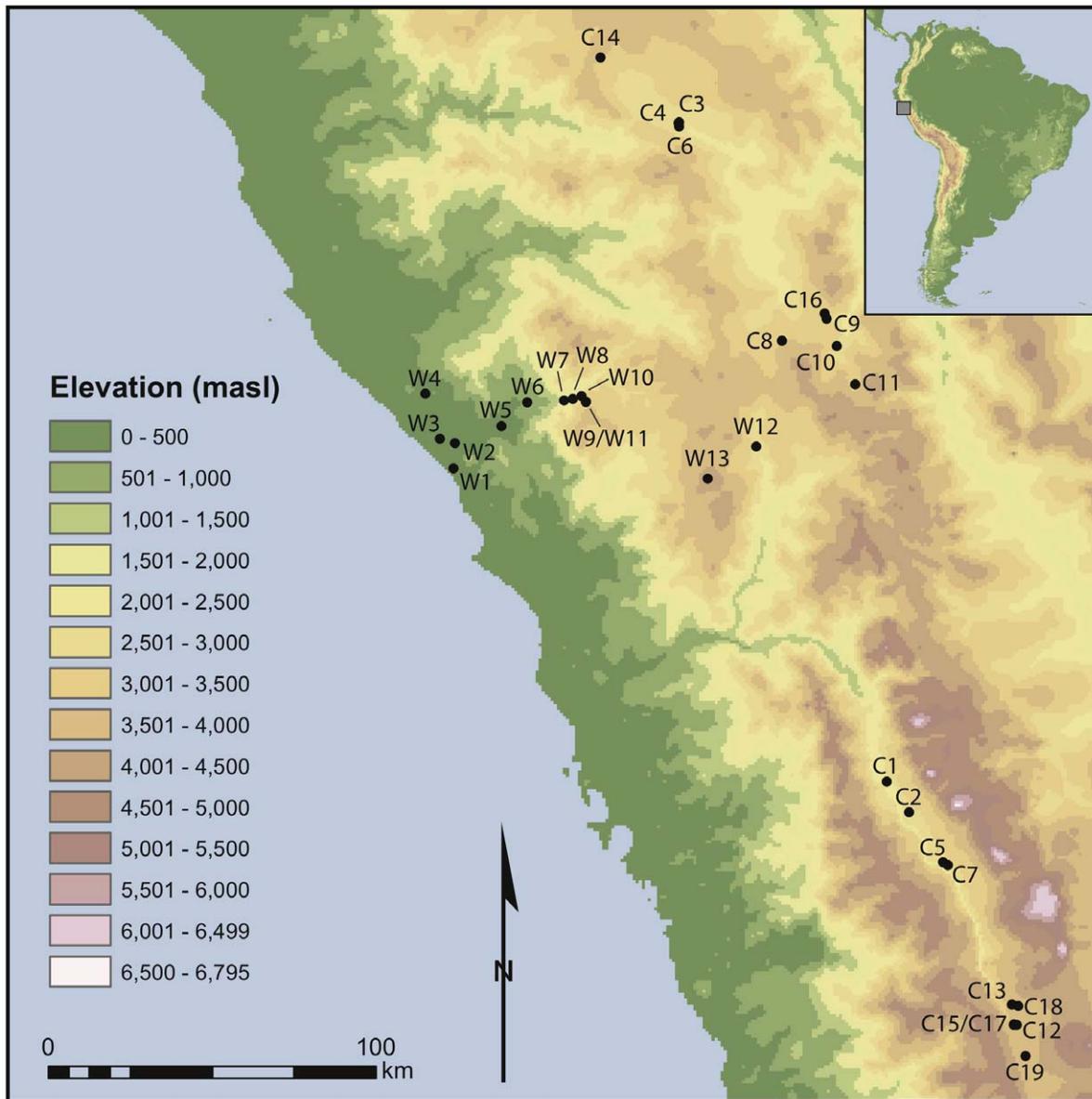
Figure 1. Digital elevation model of the study region derived from the Global 30 Arc-Second Elevation (GTOPO30) data set.
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have provided isotopic data on a much more limited scale from various sites in Argentina [17–21], Chile [22–24], Bolivia [25,26], 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.
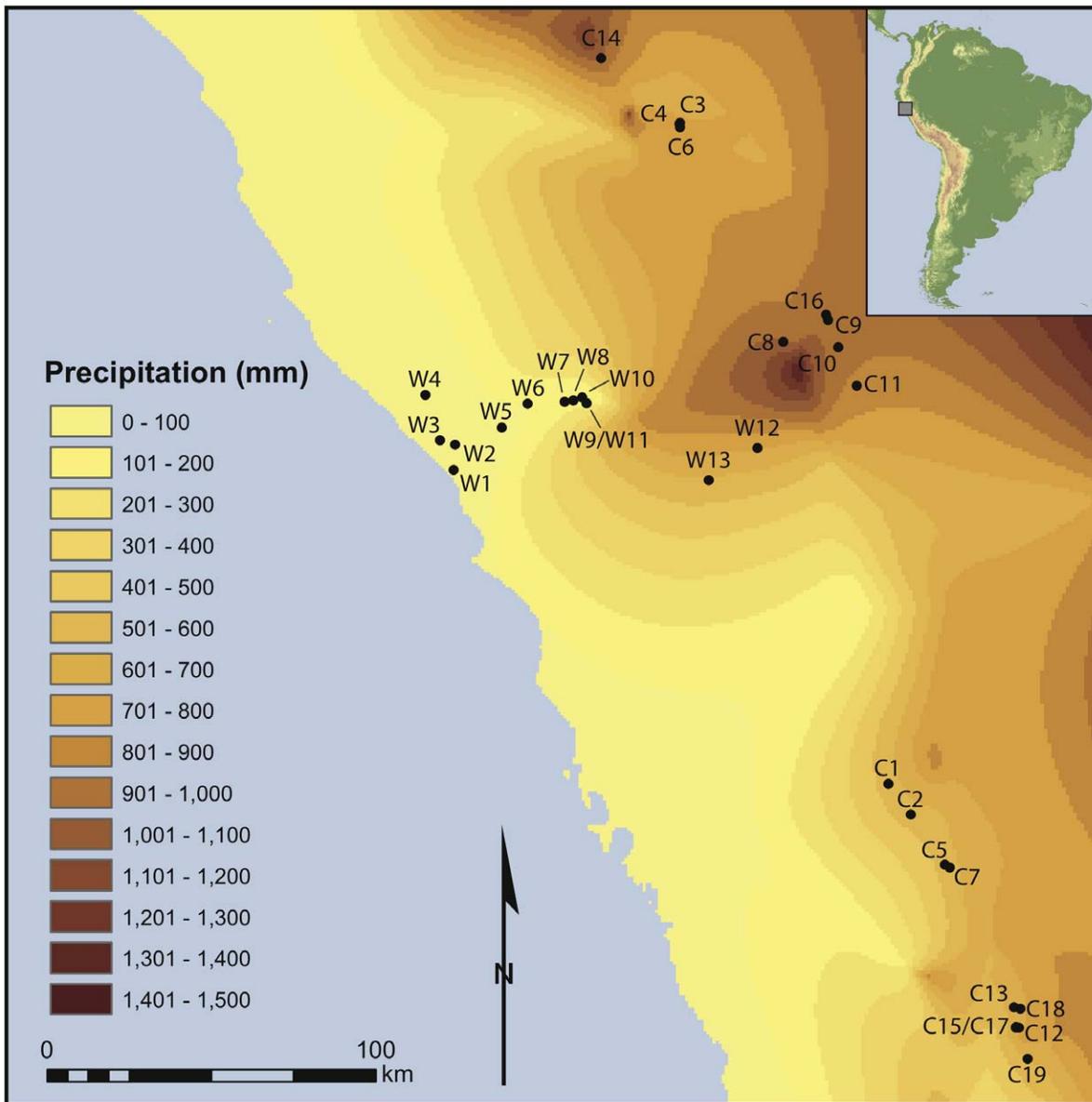
Figure 2. Extrapolated mean annual precipitation for study area. Mean annual precipitation data from 493 monitoring stations in Peru [218] were extrapolated using the natural neighbor method in ArcMap (ArcGIS 10.0, ESRI).
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The coastal region of Peru 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 2,800 masl. Again, the area is still characterized by relatively low annual precipitation, although water availability is greater close to

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*, *Polylepis*) [56]. Pastures dominated by dense bunchgrasses occur in moister areas.

Natural Variation in Plant Carbon Isotopic Composition

Photosynthetic pathway and taxonomy. The most salient mechanism influencing the carbon isotopic composition ($\delta^{13}C$) of terrestrial plants is the photosynthetic pathway utilized. Plants that fix carbon using the C_3 pathway (Calvin cycle) are characterized by lower $\delta^{13}C$ values (ca. -26 ‰) than plants utilizing the C_4 (Hatch-Slack) pathway (ca. -12 ‰) [65,66]. This is because carbon isotope discrimination ($\Delta^{13}C$) is smaller in C_4 plants than in C_3 plants. In other words, C_3 plants discriminate more strongly against the heavier isotope (^{13}C) than C_4 plants. The vast majority of C_4 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 C_4 plants are not significant, and thus a large body of research has focused on assessing and quantifying the contribution of C_4 cultigens (mostly maize, but also millet) to the diet [67]. Some desert plants and succulents exhibit carbon isotopic compositions that are intermediate between C_3 and C_4 plants. Referred to as CAM (Crassulacean acid metabolism) plants, these species fix carbon in a manner analogous to C_4 plants overnight, but utilize the C_3 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 C_3 photosynthetic pathway [69,70], but are distinct from vascular plants in that they lack stomata and CO_2 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 $\delta^{13}C$. 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 $\delta^{13}C$ values relative to canopy plants and plants growing in more open environments. Often referred to as the 'canopy effect', this is attributed to relatively ^{13}C-depleted CO_2 in the understory due to the utilization of recycled CO_2 [74–78], and/or lower irradiance and higher [CO_2] 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 posited 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 C_3 plants, with most studies finding little or no correlation between rainfall and/or water availability and plant $\delta^{13}C$ for C_4 plants [86,92]. Murphy and Bowman [87] found a positive correlation between rainfall and C_4 plant $\delta^{13}C$ over a continental (Australia) rainfall gradient, although this relationship is atypical. It is believed that the relationship between aridity and plant $\delta^{13}C$ is caused by increased stomatal closure when water availability is low, which is accompanied by decreased discrimination against ^{13}C during photosynthesis and, in turn, comparatively less negative $\delta^{13}C$ values [93,94].

Soil salinity has also been demonstrated to influence plant $\delta^{13}C$ values. In a manner somewhat analogous to drought stress, salt stress induces increased stomatal closure, and therefore reduces discrimination against ^{13}C 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 elevational gradients in plant carbon isotopic composition. Generally, foliar $\delta^{13}C$ 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 $\sim$1,000 m. The exact mechanism responsible for the relationship between plant $\delta^{13}C$ and altitude is not entirely clear. Some have suggested exceptionally high carboxylation rates relative to stomatal conductance [102,103] and/or high carboxylation efficiency [104] for plants growing at high altitudes, resulting in decreased discrimination against ^{13}C. 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 $\delta^{13}C$ values, with higher irradiance being associated with less negative $\delta^{13}C$ 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 $\delta^{13}C$ values than leaves growing in exterior, exposed areas [105,106]. These differences in $\delta^{13}C$ associated with irradiance have been attributed to differences in intercellular CO_2 concentration [94].

Intraplant and temporal variation in plant $\delta^{13}C$. Carbon isotopic composition is not necessarily equal among different plant parts. Numerous studies have observed variation in the $\delta^{13}C$ 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 ^{13}C relative to non-photosynthetic tissues, typically by 2 to 4 ‰ [108,110,111]. These differences are only consistent among C_3 plants, with C_4 plants often showing little variation between leaves and non-photosynthetic tissues, or leaves with relatively high $\delta^{13}C$ values in some cases [107,108]. There are several potential variables contributing to intraplant variation in tissue $\delta^{13}C$. First, different tissues may contain variable proportions of molecules that are relatively enriched or depleted of ^{13}C compared to total organic matter. Most notably, lipids [112] and lignin [113] are known to be characterized by relatively low $\delta^{13}C$ values, while the opposite is true for cellulose, sugars, and starches [114]. Because some studies have found significant differences in the $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ 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 ‰) 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 partly 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 animals tend to have higher $\delta^{13}C$ values than terrestrial animals, except in cases where C_4 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 C_3 and C_4 plants, with two notable exceptions. Seagrasses (*Zostera* sp.), have extremely high $\delta^{13}C$ values, typically higher than most terrestrial C_4 plants (Figure 3d). There is evidence to suggest C_4 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 $CO_{2(aq)}$ and $HCO_3^-{}_{(aq)}$ is a significant factor, as $\delta^{13}C$ of $HCO_3^-{}_{(aq)}$ is ~9 ‰ less negative than that of $CO_{2(aq)}$ [120]. Moreover, for intertidal plants, which are exposed to the atmosphere for a portion of the day, the utilization of atmospheric CO_2 further complicates matters [119]. The thickness of the diffusive boundary layer is also a potentially important factor with respect to $\Delta^{13}C$ as it may differ due to variable water velocity [121,122]. Other environmental factors have also been demonstrated to influence aquatic plant $\delta^{13}C$ values, such as: salinity [123], extracellular CO_2 concentration [124,125], light intensity [123], algal growth rate [126], water velocity [122], and water temperature [127].

Some red algae (Floridiophyceae) are characterized by consistently very low $\delta^{13}C$ values (< −30 ‰). 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 CO_2, 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 (NO_3^-) and ammonium (NH_4^+). 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 nitrogen (N_2), 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 such 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 fractionations associated with different steps in the nitrogen cycle (e.g. ammonification, nitrification, denitrification), as well as the uptake and eventual incorporation of mineralized N into organic N [135–137].

There are two important aspects of variation in N source pertinent to the present study. The first relates to N_2-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 N_2 are characterized by comparatively low $\delta^{15}N$ values, typically ~0 ‰ [27,138–140]. These plants acquire such compositions because the $\delta^{15}N$ of atmospheric N_2 is ~0 ‰ [141] and the assimilation of N from N_2-fixation is not associated with significant fractionation of ^{15}N [138–140]. By comparison, soil NO_3^- and NH_4^+ tend to have $\delta^{15}N$ values >0 ‰ [142], and non N_2-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 ‰ [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 ‰ [145,146]. The highest $\delta^{15}N$ values for animal fertilizers (>25 ‰) have been recorded for seabird guano [143,147], which consists primarily of uric acid and is subject to significant NH_4^+ volatilization. The addition of animal fertilizer N to the soil therefore adds an N source with an isotopic composition that is usually enriched in ^{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 [148,149]. 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 [12,157,158]. 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 ^{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].
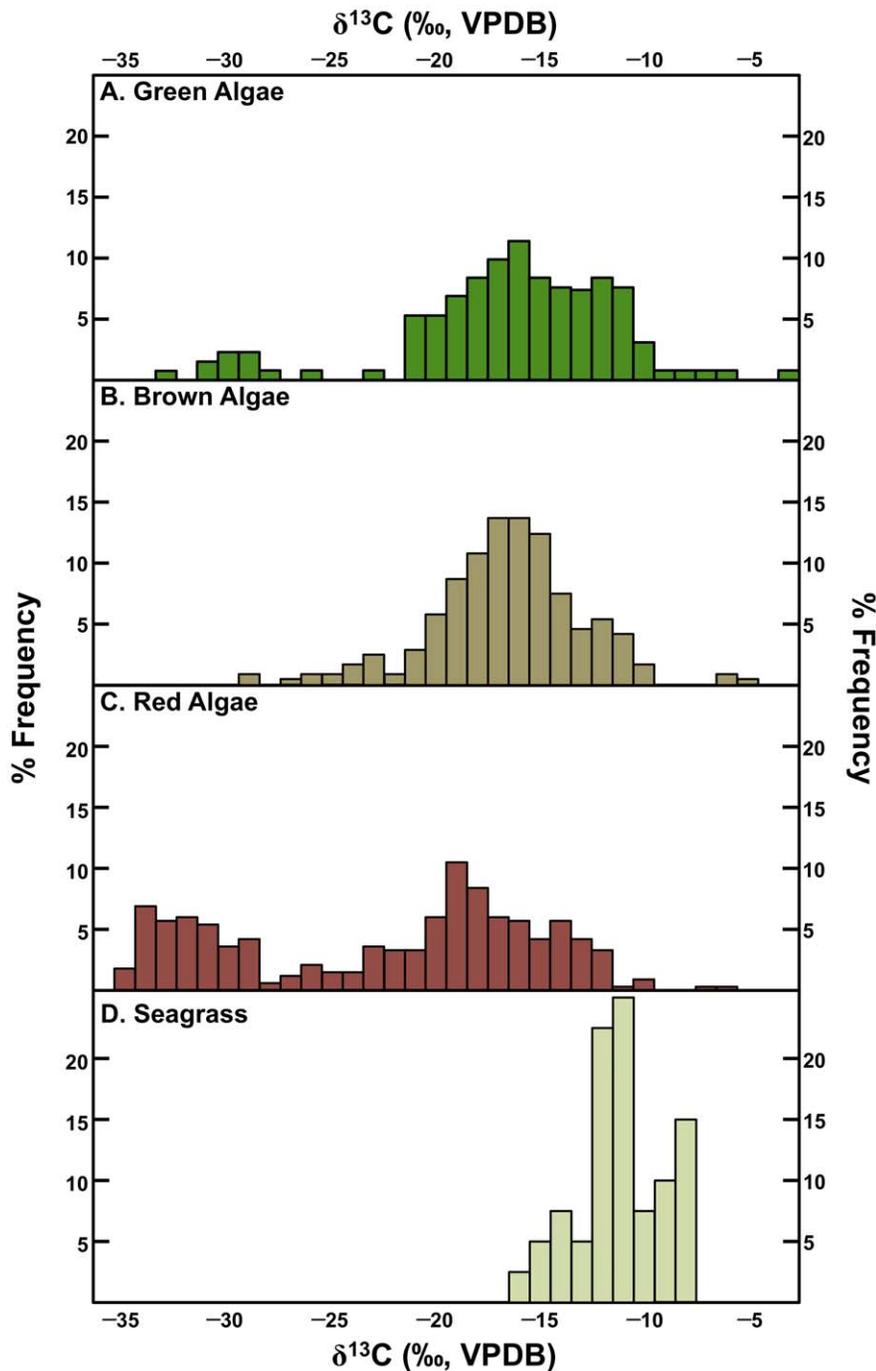
Figure 3. Frequency distributions of carbon isotopic compositions of marine macroalgae. Data are taken from published literature [119,219–235].
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Intraplant and temporal variation in plant $\delta^{15}N$. There are three main reasons that plants exhibit intraplant and temporal variation in their tissue $\delta^{15}N$ 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

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

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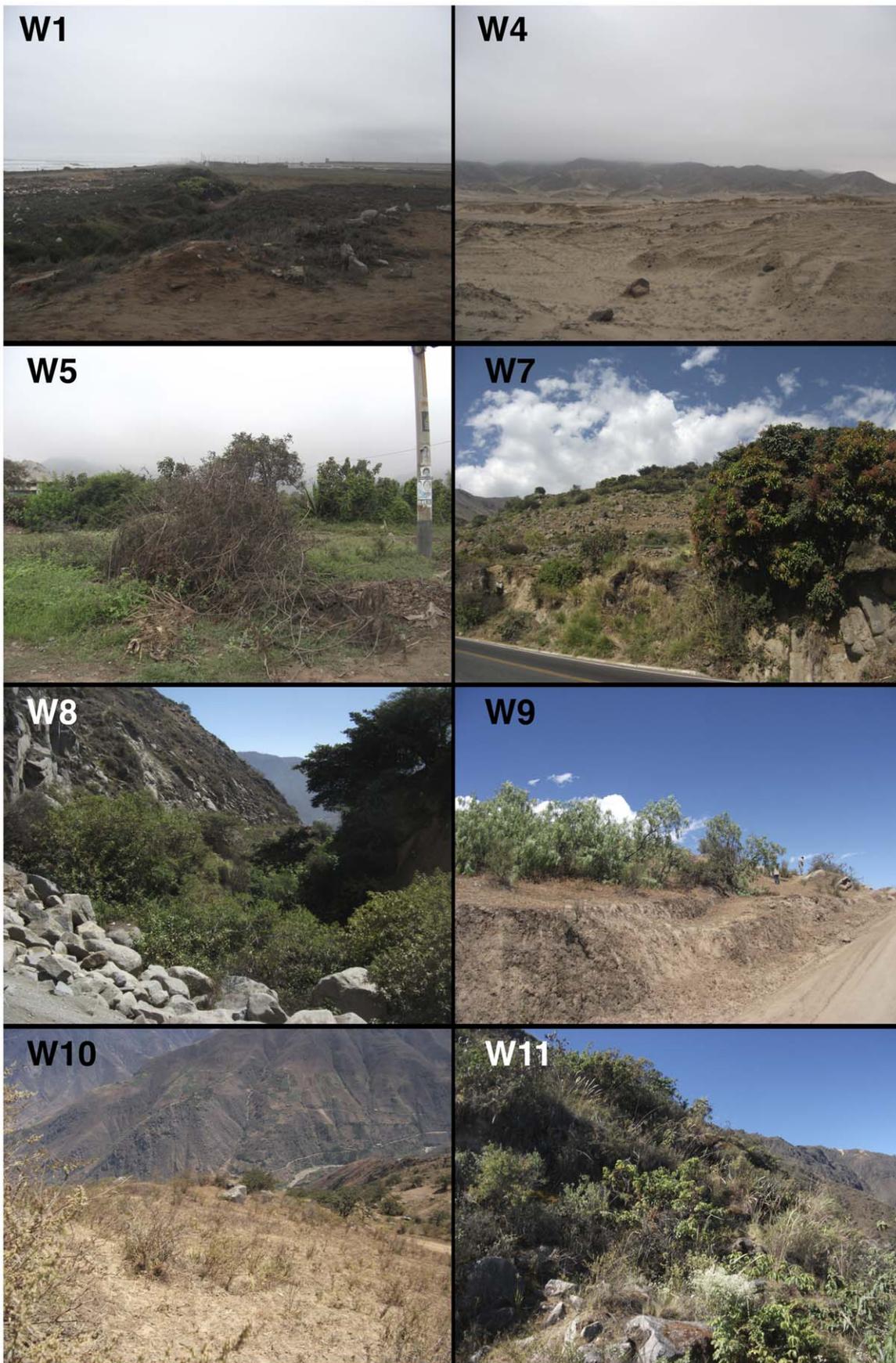
Figure 4. Images of eight of the wild plant sampling locations. Corresponding geographical data for these sites can be found in Table 6.
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the shoot has already been exposed to some fractionation associated with assimilation and is enriched in ^{15}N compared to the NO_3^- that was assimilated in the root. On this basis, it is expected that shoots will have higher δ^{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 δ^{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].

In agricultural settings, the variation within a plant over time may become particularly complex due to the application of nitrogenous fertilizers. The availability of different N-bearing species from the fertilizer (NH_4^+, NO_3^-) and the nitrogen isotopic composition of fertilizer-derived N changes over time as various soil processes (e.g. ammonification, nitrification) occur. The nature of this variation is complex and will depend on the type of fertilizer applied [147].

Environmental factors affecting plant δ^{15}N. Plant nitrogen isotopic compositions are strongly influenced by a series of environmental factors. The environmental variation in plant δ^{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 δ^{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 $\leq -0.5°C$ [12]. A large number of studies have found a negative correlation between plant δ^{15}N values and local precipitation and/or water availability. These effects have been demonstrated at regional or

Table 2. Environmental data for market plant sampling sites.

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	Huamachuco	−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	−77.9799	3471
C17	Mirador I	−9.7224	−77.4601	3477
C18	Conray Chico	−9.6705	−77.4484	3530
C19	Catac	−9.8083	−77.4282	3588

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continental [15,85–87,172,178], and global [12,176,179] scales. Several authors have hypothesized that relatively high δ^{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 δ^{15}N values
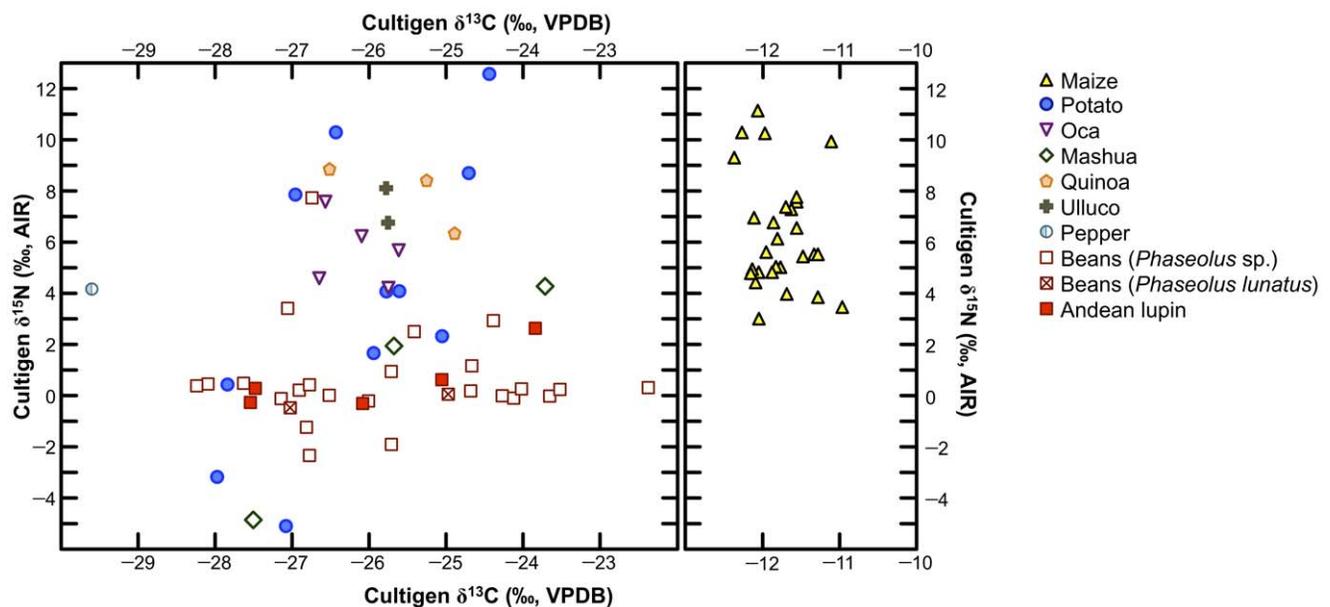
Figure 5. Carbon and nitrogen isotopic compositions of cultigens. Note that the x-axis is not continuous.
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Table 3. Mean carbon and nitrogen isotopic compositions for cultigens ($\pm 1\sigma$).

Common Name	Taxonomic Name	n	δ^{13}C (‰, VPDB)	δ^{15}N (‰, AIR)	%C	%N
Beans	*Phaseolus* sp.	24	−25.7±1.6	0.7±2.0	39.8±0.7	3.7±0.6
Beans (Lima)	*Phaseolus lunatus*	2	−26.0±1.4	−0.2±0.4	39.0±0.3	2.7±0.5
Chocho (Andean lupin)	*Lupinus mutabilis*	5	−26.0±1.6	0.6±1.2	48.3±2.8	6.8±1.3
Coca	*Erythroxylum coca*	4	−29.8±0.9	–	45.4±1.5	–
Maize (Grain)	*Zea mays*	27	−11.8±0.4	6.4±2.2	40.4±0.5	1.2±0.2
Maize (Leaf)	*Zea mays*	2	−12.9±0.4	4.5±1.6	41.9±4.6	1.3±1.3
Mashua	*Tropaeolum tuberosum*	3	−25.6±1.9	0.5±4.7	41.5±2.8	3.0±0.7
Oca	*Oxalis tuberosa*	6	−26.4±0.7	5.7±1.3	43.1±3.2	1.6±0.6
Pepper	*Capsicum annuum*	1	−29.6	4.2	48.3	2.1
Potato	*Solanum tuberosum*	12	−26.3±1.3	4.0±5.5	40.5±1.5	1.4±0.4
Quinoa	*Chenopodium quinoa*	3	−25.6±0.9	7.9±1.3	39.9±2.1	2.6±0.3
Ulluco	*Ullucus tuberosus*	2	−25.8±0.0	7.5±1.0	40.6±0.4	3.4±1.0

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

The nature of the relationship between rainfall and plant δ^{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 δ^{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 δ^{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 δ^{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. Entire large cultigens (e.g.

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

Cultigen %N	Bean (*P. lunatus*)	Andean lupin	Maize	Mashua	Oca	Potato	Quinoa	Ulluco
Bean (*Phaseolus* sp.)	0.860	1.000	**<0.001**	1.000	**0.003**	0.798	**0.028**	0.880
Bean (*P. lunatus*)	–	0.971	**<0.001**	1.000	**0.005**	0.479	**0.037**	0.121
Andean lupin	–	–	**<0.001**	1.000	**0.006**	0.802	**0.020**	0.060
Maize	–	–	–	0.696	1.000	0.983	0.855	0.917
Mashua	–	–	–	–	0.788	1.000	0.626	0.723
Oca	–	–	–	–	–	1.000	0.626	0.723
Potato	–	–	–	–	–	–	0.688	0.780
Quinoa	–	–	–	–	–	–	–	1.000

Values in boldface are statistically significant ($p<0.05$).
doi:10.1371/journal.pone.0053763.t004
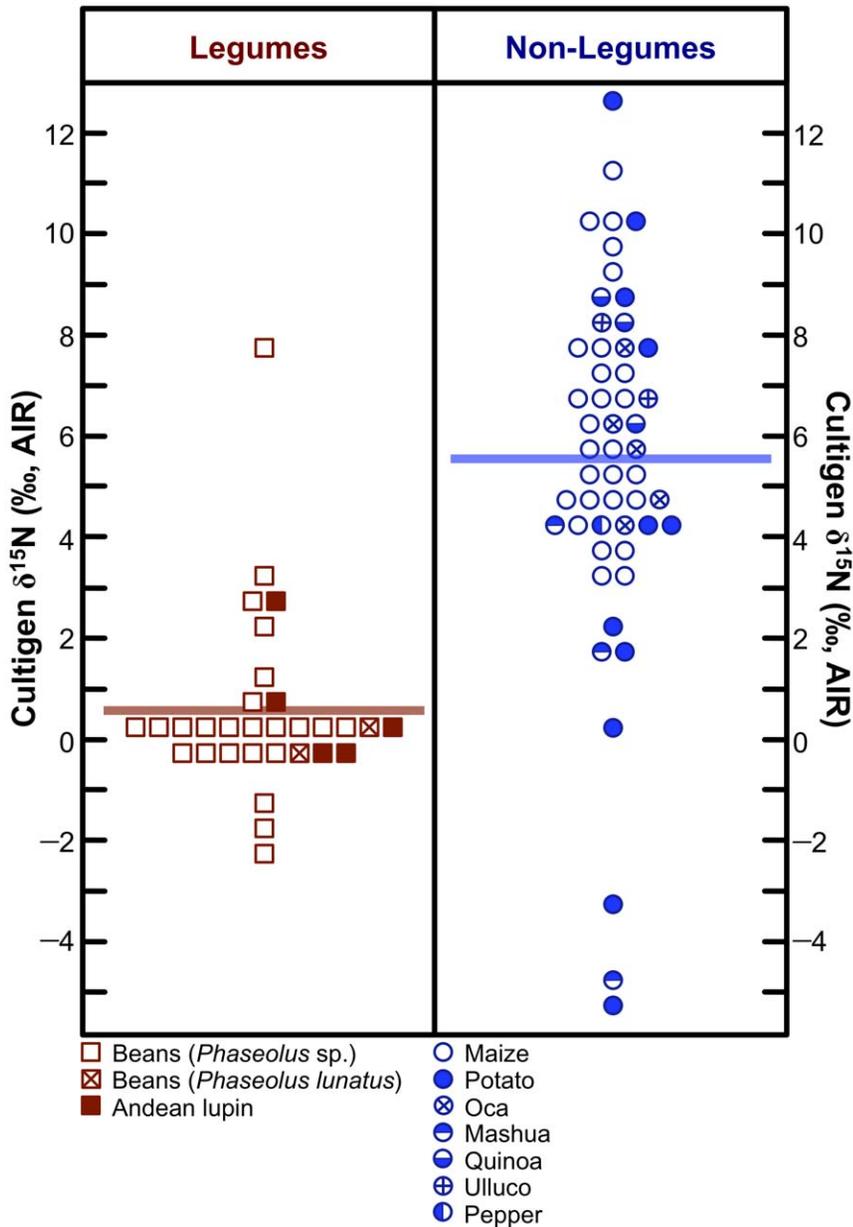
Figure 6. Dot-matrix plot of nitrogen isotopic compositions of legumes and non-legumes. Horizontal bars represent means. Increment = 0.5 ‰.
doi:10.1371/journal.pone.0053763.g006

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-Or-meaux, 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
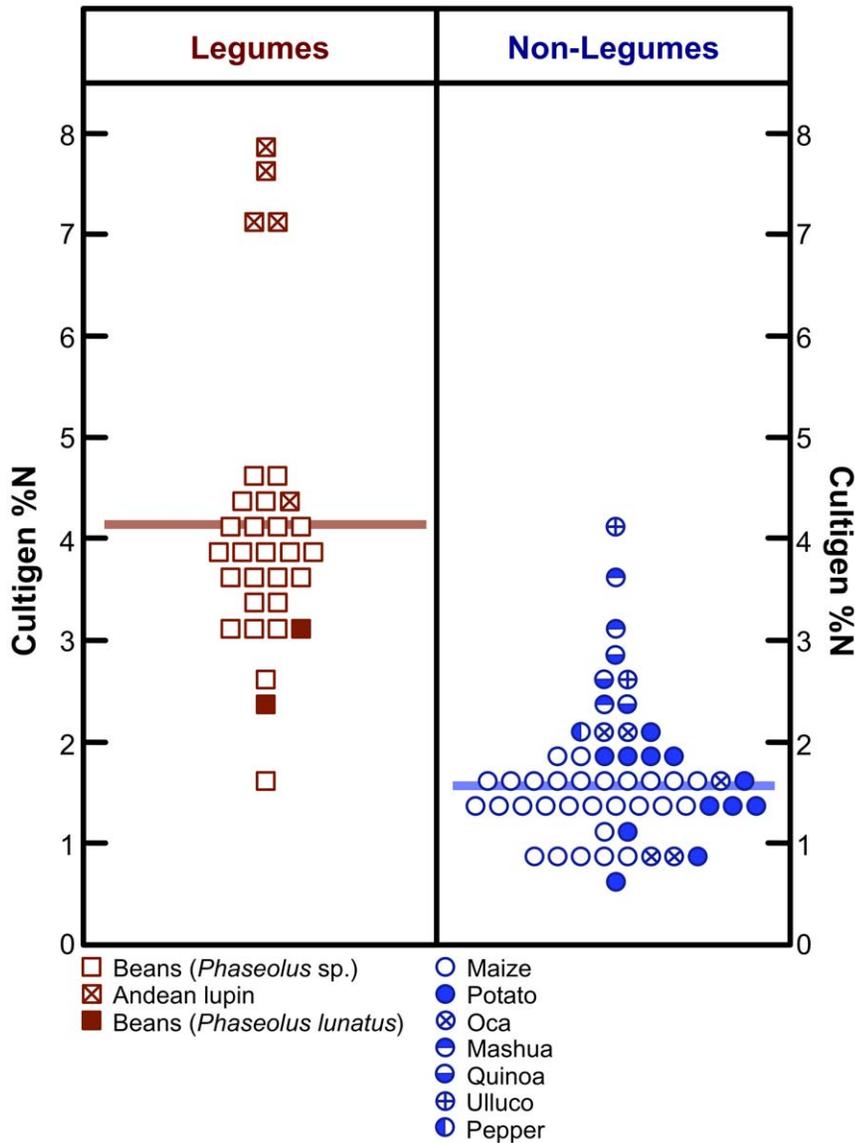
Figure 7. Dot-matrix plot of nitrogen content of legumes and non-legumes. Horizontal bars represent means. Increment = 0.25%.
doi:10.1371/journal.pone.0053763.g007

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 (δ^{13}C and δ^{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 CO_2 being removed with a Carbo-Sorb trap (Elemental Microanalysis, Okehampton, Devon, UK) prior to isotopic analysis.

Sample δ^{13}C and δ^{15}N values were calibrated to VPDB and AIR, respectively, with USGS40 (accepted values: δ^{13}C = −26.39 ‰, δ^{15}N = −4.52 ‰) and USGS41 (accepted values: δ^{13}C = 37.63 ‰,

δ^{15}N = 47.6 ‰). 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 δ^{13}C value of −24.03±0.14 ‰ was obtained for 81 analyses of the internal keratin standard, which compared well with its average value of −24.04 ‰. A δ^{13}C value of −10.46±0.09 ‰ was obtained for 46 analyses of IAEA-CH-6, which compared well with its accepted value of −10.45 ‰. Sample reproducibility was ±0.10 ‰ for δ^{13}C and ±0.50% for %C (50 replicates). A δ^{15}N value of 6.37±0.13 ‰ was obtained for 172 analyses of an internal keratin standard, which compared well with its average value of 6.36 ‰. A δ^{15}N value of 20.3±0.4 ‰ was obtained for 76 analyses of IAEA-N-2, which compared well with its accepted value of 20.3 ‰. Sample reproducibility was ±0.14 ‰ for δ^{15}N and ±0.10% 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

Table 5. Results of ANOVA post-hoc tests (Dunnett's T3) for cultigen N content.

Cultigen %N	Bean (*P. lunatus*)	Andean lupin	Maize	Mashua	Oca	Potato	Quinoa	Ulluco
Bean (*Phaseolus* sp.)	0.637	0.072	**<0.001**	0.869	**0.009**	**<0.001**	0.123	1.000
Bean (P. lunatus)	–	**0.037**	0.462	1.000	0.619	0.505	1.000	0.995
Andean lupin	–	–	**0.009**	**0.034**	**0.005**	**0.008**	**0.021**	0.295
Maize	–	–	–	0.232	0.981	0.992	0.101	0.566
Mashua	–	–	–	–	**0.019**	1.000	0.216	**0.009**
Oca	–	–	–	–	–	1.000	**0.033**	0.001
Potato	–	–	–	–	–	–	**0.033**	0.001
Quinoa	–	–	–	–	–	–	–	0.885

Values in boldface are statistically significant (*p*<0.05).
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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 C_3 and C_4 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 analyses and 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 $\delta^{13}C$ and $\delta^{15}N$ 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 $\delta^{13}C$ values for C_3 cultigens ranged from -29.8 ± 0.9 ‰ (coca) to -25.6 ± 1.9 ‰ (mashua). The mean $\delta^{13}C$ value for maize, which was the only C_4 plant examined, was -11.8 ± 0.4 ‰. Mean $\delta^{15}N$ values for cultigens were typically more variable than $\delta^{13}C$ 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 $\delta^{13}C$ among cultigens ($F_{[7,49]}=0.3$, $p=0.93$), but there were for $\delta^{15}N$ (maize included) ($F_{[8,73]}=9.7$, $p<0.001$). Results of post-hoc Dunnett's T3 test for $\delta^{15}N$ differences among individual cultigen species are presented in Table 4. The three leguminous species were generally characterized by significantly lower $\delta^{15}N$ values than non-leguminous species (Table 4); collectively, legumes were characterized by significantly lower $\delta^{15}N$ values than non-legumes (Figure 6; $F_{[1,80]}=51.8$, $p<0.001$).

Table 6. Environmental data for wild plant sampling sites and summary of number of C_3 and C_4 plant species sampled.

Site ID	Site Name	Latitude	Longitude	Altitude (masl)	MAP (mm)[1]	C_3 Plant Taxa Sampled	C_4 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 Chuco	−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.
doi:10.1371/journal.pone.0053763.t006

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

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 $\delta^{15}N$ (‰)
Eriochloa mutica	W1	10	Grass	−11.6	−1.5	−11.7	1.6	–	–	–	–	−12.1	−0.2
Distichia spicata	W1	10	Grass	−14.9	−3.2	–	–	–	–	–	–	–	–
Baccharis glutinosa	W1	10	Shrub	−27.4	3.3	−26.6	5.1	–	–	−27.0	4.1	−26.9	4.2
Rauvolfia sp.	W1	10	Shrub	−28.0	9.8	−27.9	11.0	–	–	–	–	–	–
Plantago major[1]	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 crispata[1]	W1	10	Herb	−29.8	13.7	−30.4	13.7	−30.5	11.6				
Rosippa nastrutium aquaticum[1]	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 peruviana	W2	33	Herb	−29.6	2.2	−30.0	1.7	–	–	–	–	–	–
Arundo donax[1]	W2	33	Grass	−30.3	8.5	−30.1	10.2	–	–	–	–	–	–
Acacia huarango[2]	W2	33	Shrub	−31.0	3.5	−30.0	2.3	–	–	−29.8	3.4	–	–
Psittacanthus obovatus	W2	33	Shrub (Parasitic)	−31.9	5.1	−30.6	6.2						
Prosopis pallida[2]	W3	38	Tree	−27.9	4.0	−28.9	1.5	–	–	−29.1	5.8	–	–
Acacia macracantha[2]	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 pyriformis	W4	164	Shrub	−22.5	10.3	−22.2	10.4	–	–	–	–	−22.4	12.1
Trixis cacalioides	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	–	–	–	–	–	–
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 subfusiformis	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 δ13C (‰)	Leaf δ15N (‰)	Stem δ13C (‰)	Stem δ15N (‰)	Root δ13C (‰)	Root δ15N (‰)	Flowers δ13C (‰)	Flowers δ15N (‰)	Seeds δ13C (‰)	Seeds δ15N (‰)
Cyperus corymbosus	W6	447	Sedge	−13.1	8.3	−11.2	8.8	−11.7	7.8	−14.2	9.1	—	—
Echinochloa crusgalli[1]	W6	447	Grass	−13.4	2.8	−13.8	2.8	—	—	—	—	−13.7	3.9
Cynodon dactylon[1]	W6	447	Grass	−13.9	0.8	—	—	—	—	−14.1	1.2	—	—
Sorghum halepense[1]	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	—	—	—	—	—	—
Amaranthus spinosus	W6	447	Herb	−14.4	13.3	−13.8	16.1	—	—	−14.0	15.3	−14.4	15.3
Gynerium 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[2]	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	—	—	—	—	−26.9	9.8
Crotalaria incae[2]	W6	447	Shrub	−27.3	0.2	−26.6	−2.4	—	—	−25.4	1.0	−26.0	−0.8
Ludwigia octovalvis[2]	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 latilofolia	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	—	—
Verbena littoralis	W6	447	Herb	−28.8	7.9	−28.4	5.8	—	—	—	—	−27.7	7.3
Cyperus 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[1]	W7	1181	Grass	−13.3	5.6	−13.4	7.3	—	—	—	—	−14.5	3.1
Cenchrus 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 microcalyx	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
Asclepias 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	—	—	—	—	—	—
Centaurea melitensis	W8	1557	Herb	−29.5	0.5	−29.8	0.2	—	—	—	—	−28.9	1.7
Mentzelia 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	—	—

Table 7. Cont.

Taxonomic Name	Site ID	Altitude	Type	Leaf δ¹³C (‰)	Leaf δ¹⁵N (‰)	Stem δ¹³C (‰)	Stem δ¹⁵N (‰)	Root δ¹³C (‰)	Root δ¹⁵N (‰)	Flowers δ¹³C (‰)	Flowers δ¹⁵N (‰)	Seeds δ¹³C (‰)	Seeds δ¹⁵N (‰)
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.[2]	W9	2150	Herb	−27.1	1.4	−27.1	3.4	–	–	−26.6	3.2	−26.7	0.8
Alonsoa meridionalis	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	–	–
Polypogon sp.	W9	2150	Grass	−31.1	−5.3	−27.9	−4.4	−31.0	2.4	−27.8	−3.7	–	–
Browallia 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
Caesalpina spinosa[2]	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[1]	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 purpurem[1]	W11	2947	Grass	−12.5	7.2	−12.8	6.6	–	–	–	–	−15.5	7.1
Ruellia floribunda	W11	2947	Herb	−23.7	4.5	−24.0	1.9	–	–	−23.5	4.9	–	–
Schinus molle	W11	2947	Tree	−24.6	2.3	−23.4	0.3	–	–	−21.3	0.8	–	–
Spartium junceum[1,2]	W11	2947	Shrub	−26.5	1.1	−27.1	−1.1	–	–	−23.7	−1.3	−25.4	0.8
Acacia aroma[2]	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	–	–	–	–	–	–
Leonotis nepetifolia[1]	W11	2947	Shrub	−28.0	2.2	–	–	–	–	−27.2	3.0	−26.1	2.0
Lycianthes lycioides	W11	2947	Shrub	−28.0	−0.3	–	–	–	–	–	–	−24.3	2.0
Phenax hirtus	W11	2947	Shrub	−28.3	2.5	−29.1	7.1	–	–	–	–	−28.5	6.9
Inga feulleu[2]	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 δ¹³C (‰)	Leaf δ¹⁵N (‰)	Stem δ¹³C (‰)	Stem δ¹⁵N (‰)	Root δ¹³C (‰)	Root δ¹⁵N (‰)	Flowers δ¹³C (‰)	Flowers δ¹⁵N (‰)	Seeds δ¹³C (‰)	Seeds δ¹⁵N (‰)
Andropogon sp.	W12	3041	Grass	−13.5	−1.6	–	–	−13.2	−1.0	–	–	–	–
Sebastiania obtusifolia	W12	3041	Shrub	−23.7	0.8	−24.7	0.0	–	–	–	–	−24.0	2.5
Lupinus aridulus[2]	W12	3041	Herb	−24.3	2.0	−23.7	2.2	–	–	−22.7	4.0	−22.0	5.4
Silybum marianum[1]	W12	3041	Herb	−25.9	2.2	−25.8	1.6	–	–	–	–	−25.1	2.2
Phrygilanthus sp.	W12	3041	Shrub (Parasitic)	−25.9	−0.5	−24.7	7.3	–	–	–	–	–	–
Solanum amotapense	W12	3041	Shrub	−25.9	7.9	−25.2	5.1	–	–	–	–	−24.7	8.3
Acacia sp.[2]	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	1.0	–	–
Aristida adsensionis	W12	3041	Grass	−26.5	−2.6	−26.2	−2.0	–	–	−26.7	−1.0	–	–
Baccharis emarginata	W12	3041	Shrub	−26.5	−0.2	−25.4	0.1	–	–	–	–	–	–
Brassica campestris	W12	3041	Herb	−27.1	2.3	–	–	–	–	–	–	−25.4	4.3
Mauria sp.	W12	3041	Tree	−27.2	6.1	−25.8	3.1	–	–	–	–	–	–
Solanum agrimoniaefolium	W12	3041	Shrub	−28.0	6.0	−28.2	3.8	–	–	–	–	−26.6	4.1
Salvia punctata	W12	3041	Herb	−28.1	−3.5	–	–	–	–	−27.7	−2.1	−27.3	−1.7
Duranta sp.	W12	3041	Shrub	−28.4	1.3	−27.6	0.8	–	–	–	–	–	–
Flourensia cajabambensis	W12	3041	Shrub	−28.6	2.9	−27.5	2.7	–	–	–	–	−29.4	2.5
Marrubium vulgare	W12	3041	Herb	−28.8	3.8	−26.6	1.4	−27.9	1.0	−26.9	4.0	–	–
Scutellaria sp.	W12	3041	Herb	−28.8	2.2	−28.6	0.6	–	–	–	–	–	–
Viguiera peruviana	W12	3041	Shrub	−28.9	5.3	−27.3	4.6	–	–	–	–	−26.6	5.4
Jungia rugosa	W12	3041	Shrub	−28.9	1.4	−26.8	1.1	–	–	−27.1	2.7	–	–
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	–	–	–	–	–	–
Usnea andina	W13	4070	Lichen	−20.5	−6.5	–	–	–	–	–	–	–	–
Astragalus garbancillo[2]	W13	4070	Shrub	−24.6	4.2	−25.3	3.0	−25.1	3.8	−23.8	3.9	−22.5	5.4
Luzula sp.	W13	4070	Sedge	−25.1	0.9	–	–	−25.0	3.9	−25.1	3.2	–	–
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	9.4	–	–	−26.6	11.9
Agrostis breviculmis	W13	4070	Grass	−25.9	2.1	–	–	−26.0	4.1	−25.5	2.4	–	–
Chuquiraga spinosa	W13	4070	Shrub	−26.0	−0.5	−24.9	−1.1	−24.4	−1.4	−24.3	−0.7	−24.4	−0.2
Werneria nubigena	W13	4070	Herb	−26.2	1.3	–	–	−25.8	1.8	–	–	–	–
Festuca dolichophylla	W13	4070	Grass	−26.3	−1.8	–	–	−25.4	−0.3	–	–	−26.5	3.6
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	–	–	–	–

Table 7. Cont.

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 $\delta^{15}N$ (‰)
Stipa mucronata	W13	4070	Grass	−27.7	−1.4	–	–	−26.2	1.5	–	–	−26.5	1.5
Stenandrium dulce	W13	4070	Herb	−28.4	0.6	–	–	–	–	–	–	–	–
Senecio nutans	W13	4070	Shrub	−29.4	6.2	−28.6	5.0	−27.6	5.3	–	–	–	–

1. Species is invasive or introduced.
2. Member of the family Fabaceae (legume).
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Cultigen N content is presented in Table 3 and Figure 7. Mean %N for cultigens ranged from $1.2\pm0.2\%$ (maize) to $6.8\pm1.3\%$ (Andean lupin). Results of post-hoc Dunnett's T3 test for differences between individual cultigen 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; $F_{[1,80]} = 116.0$, $p < 0.001$).

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 C_4 plant taxa was generally higher at lower altitude sites receiving low amounts of rainfall. This fits with what is known about the global distribution of C_4 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 $\delta^{13}C$ values for C_3 plants ranged from -31.9 to -22.5 ‰, with a mean value of -27.6 ± 1.9 ‰ ($n = 122$). Foliar $\delta^{13}C$ values for C_4 plants ranged from -15.6 to -11.6 ‰, with a mean value of -13.5 ± 1.0 ‰ ($n = 17$). Foliar $\delta^{15}N$ values for C_3 plants ranged from -4.1 to 13.0‰, with a mean value of 3.7 ± 4.0 ‰. Foliar $\delta^{15}N$ values for C_4 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 $\delta^{13}C$ value intermediate between C_3 and C_4 plants (-20.5 ‰) and a very low $\delta^{15}N$ value (-6.5 ‰), consistent with previously reported results for lichens [71–73].

There were no significant differences in foliar $\delta^{15}N$ among plant functional groups ($F_{[3,132]} = 1.8$, $p = 0.15$). Foliar $\delta^{13}C$ differed significantly among plant functional groups ($F_{[5,130]} = 195.0$, $p < 0.001$), although this was driven by differences between C_3 and C_4 groups; there were no significant differences in foliar $\delta^{13}C$ between plant functional groups within C_3 and C_4 groups (Table 8).

There was no clear pattern of intraplant variation in $\delta^{15}N$ (Figure 8) with differences in $\delta^{15}N$ between tissues ($\Delta^{15}N$) being highly variable: $\Delta^{15}N_{stem-leaf} = -0.3\pm2.3$ ‰, $\Delta^{15}N_{root-leaf} = 0.4\pm3.1$ ‰, $\Delta^{15}N_{flower-leaf} = 0.5\pm1.4$ ‰, $\Delta^{15}N_{seed-leaf} = 0.5\pm1.7$ ‰. Conversely, foliar tissue was typically characterized by lower $\delta^{13}C$ values than all other tissues analyzed (Figure 9), and intraplant variation was generally smaller: $\Delta^{13}C_{stem-leaf} = 0.5\pm0.9$ ‰, $\Delta^{13}C_{root-leaf} = 0.4\pm0.8$ ‰, $\Delta^{13}C_{flower-leaf} = 0.6\pm1.0$ ‰, $\Delta^{13}C_{seed-leaf} = 0.5\pm1.7$ ‰. For C_4 plants ($n = 17$), there was no clear pattern of intraplant variation in $\delta^{13}C$: $\Delta^{13}C_{stem-leaf} = 0.0\pm0.8$ ‰, $\Delta^{13}C_{root-leaf} = 0.5\pm0.7$ ‰, $\Delta^{13}C_{flower-leaf} = -0.3\pm0.6$ ‰, $\Delta^{13}C_{seed-leaf} = -0.2\pm1.3$ ‰.

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 $\delta^{15}N$ values ranged from -1.0 to 9.6 ‰, suggesting that some species are not engaged in active N_2-fixation. While wild legumes were characterized by lower foliar $\delta^{15}N$ 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 C_3 plant foliar $\delta^{13}C$ and $\delta^{15}N$ 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 $\delta^{15}N$ values at low altitude

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$_3$ 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$_3$ Grass/Sedge	–	**<0.001**	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 ($p<0.05$).
doi:10.1371/journal.pone.0053763.t008

sites were 2 to 8 ‰ higher than mean foliar $\delta^{15}N$ values at high altitude sites. Foliar $\delta^{15}N$ was negatively correlated with mean annual precipitation (Spearman's $\rho = -0.770$, $p = 0.009$) and altitude (Spearman's $\rho = -0.782$, $p = 0.008$). Foliar $\delta^{13}C$ was positively correlated with mean annual precipitation (Spearman's $\rho = 0.879$, $p = 0.001$) and altitude (Spearman's $\rho = 0.903$, $p<0.001$). 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 ± 0.7 ‰ (*Gymnogongrus furcellatus*) to -14.2 ± 1.2 ‰ (*Grateloupia doryphora*). Mean $\delta^{15}N$ values for marine plants ranged from 2.5 ± 0.9 ‰

(*Gymnogongrus furcellatus*) to 7.8 ± 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 ~ 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
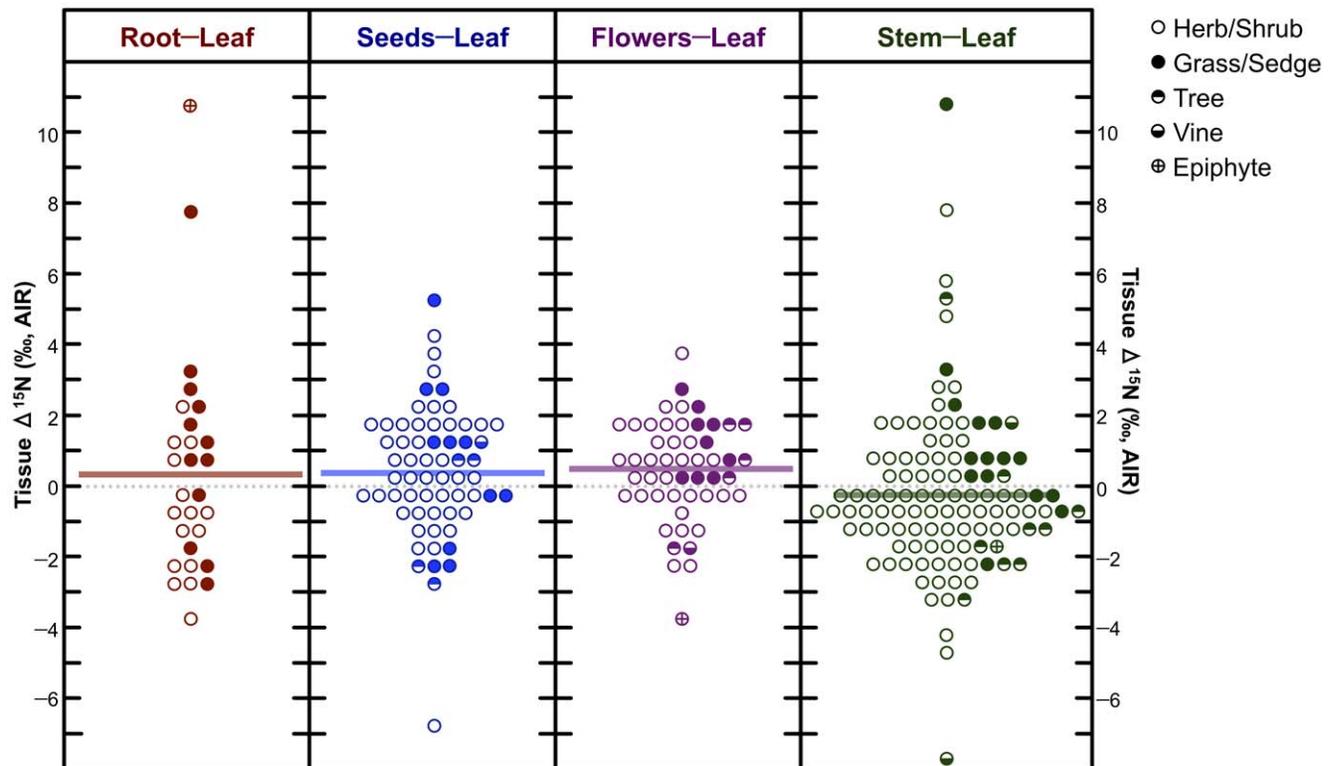
Figure 8. Dot-matrix plot of differences in nitrogen isotopic composition between foliar and other tissues ($\Delta^{15}N$). Horizontal bars represent means. Increment = 0.5 ‰.
doi:10.1371/journal.pone.0053763.g008
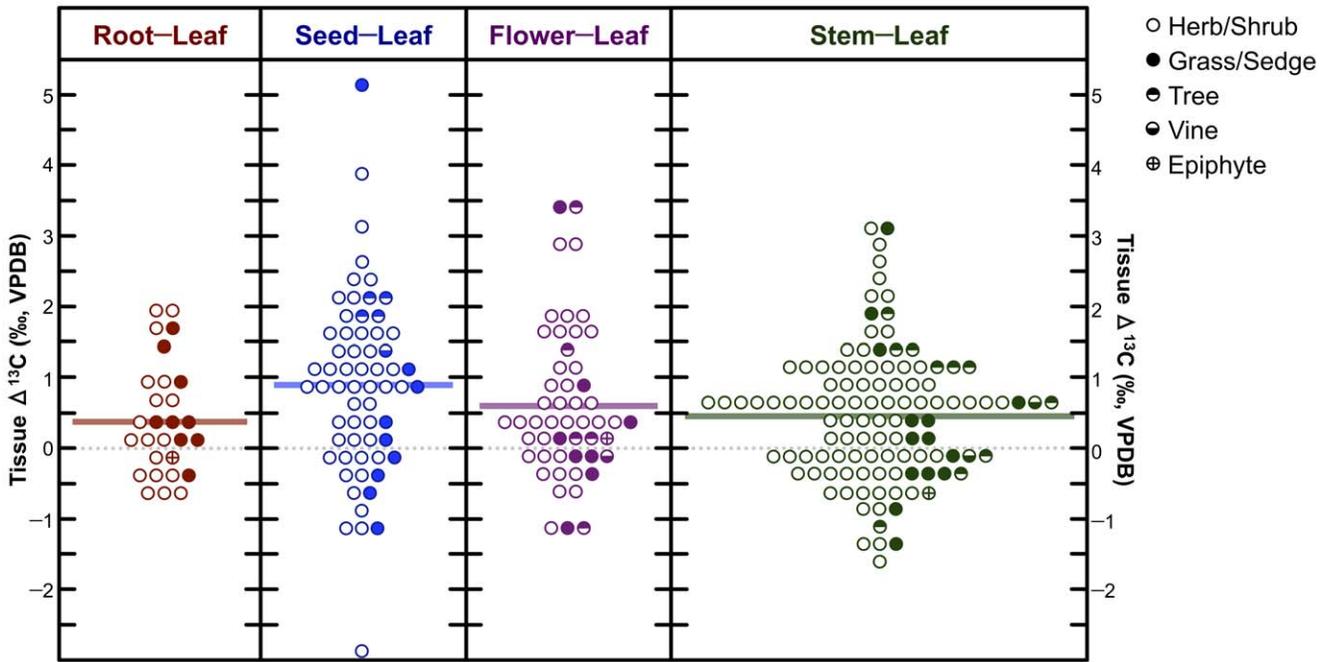
Figure 9. Dot-matrix plot of differences in carbon isotopic composition between foliar and other tissues (Δ^{13}C). Horizontal bars represent means. Increment = 0.5 ‰.
doi:10.1371/journal.pone.0053763.g009

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

For the most part, the δ^{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 δ^{15}N values in legumes in that region is tenuous. Where the potential effects of nitrogenous fertilizers on legume δ^{15}N values are unknown (as is the case for the data presented by Warriner et al. [187]), the interpretation of δ^{15}N values in legumes and

Table 9. Mean ($\pm 1\sigma$) isotopic and elemental compositions for sampling locations with >3 plant species sampled (data for C$_3$ plants only).

Site ID	Latitude	Longitude	Altitude (masl)	MAP (mm)[1]	n[2]	δ^{13}C (‰, VPDB)	δ^{15}N (‰, AIR)
W1	−8.1956	−78.9996	10	7	7	−29.1±1.2	7.9±4.5
W2	−8.1267	−78.9963	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.
doi:10.1371/journal.pone.0053763.t009
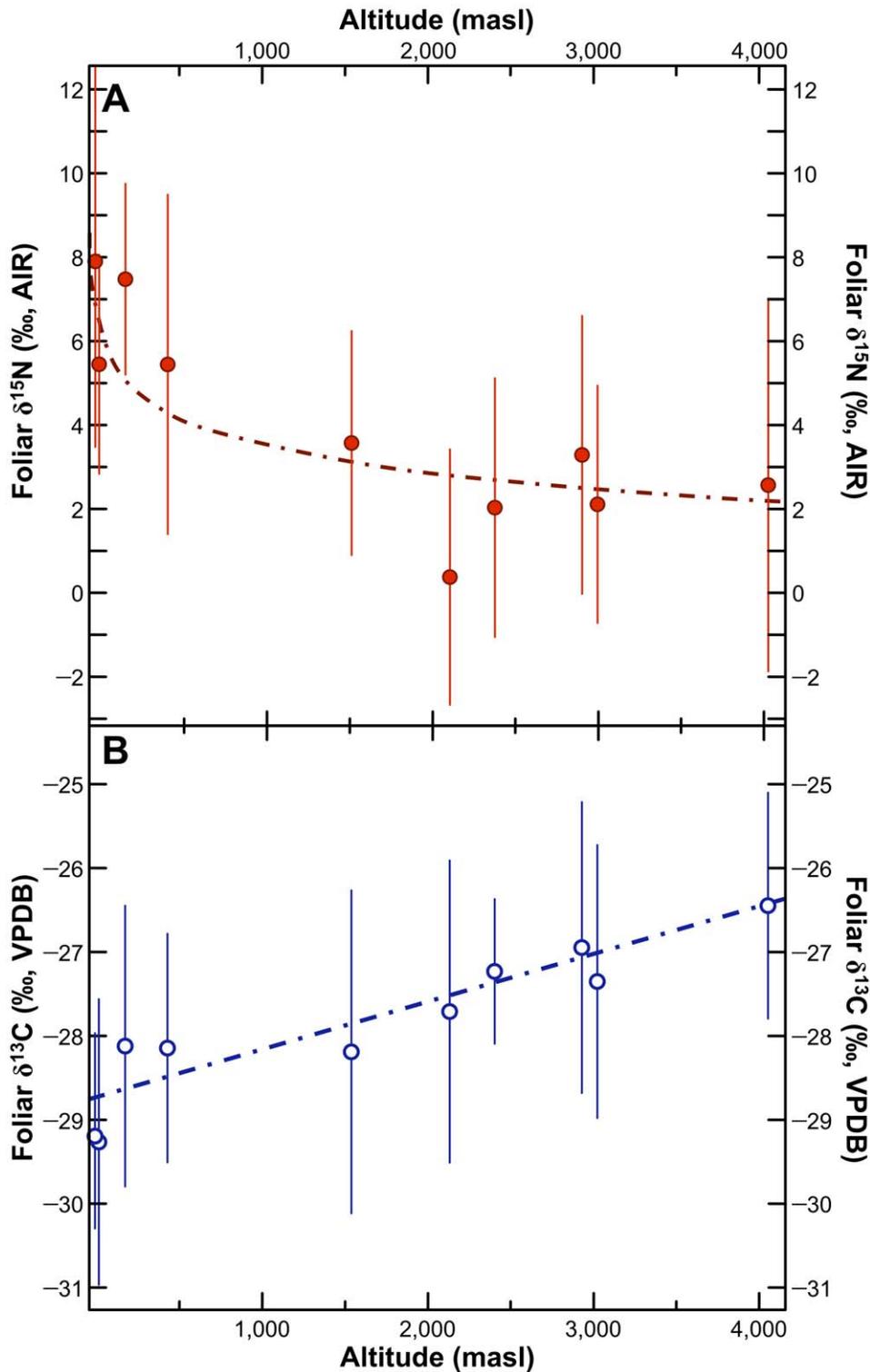
Figure 10. Bivariate plots of foliar $\delta^{15}N$ and altitude (A) and foliar $\delta^{13}C$ (B) for C_3 plants only. Points represent means $\pm 1\sigma$ for sites with ≥ 5 C_3 plant species sampled. Equation for $\delta^{15}N$ and altitude: $y = 10.3 - \log x$, $r^2 = 0.71$; $p = 0.002$. Equation for $\delta^{13}C$ and altitude: $y = x/1{,}733 - 28.8$, $r^2 = 0.85$; $p < 0.001$.
doi:10.1371/journal.pone.0053763.g010

non-legumes is not straightforward. While there was some overlap in $\delta^{15}N$ values between legumes and non-legumes in this study, leguminous cultigens had significantly higher N contents (Figure 7; Table 5) and significantly lower $\delta^{15}N$ values (Figure 6; Table 4) than non-legumes.

Aside from the differences in $\delta^{15}N$ between legumes and non-legumes, it is very difficult to generalize the $\delta^{15}N$ values for
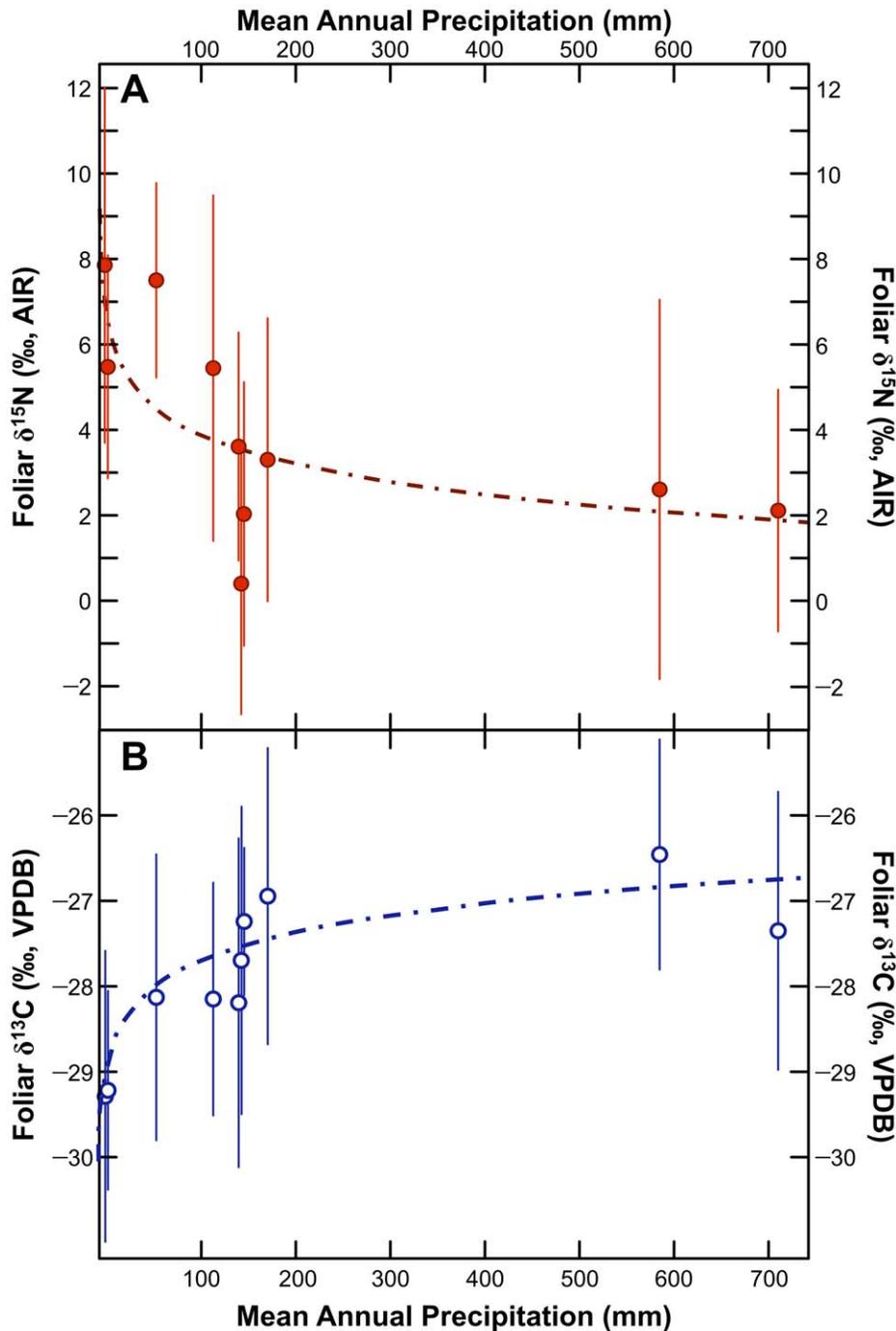
Figure 11. Bivariate plots of foliar δ^{15}N and mean annual precipitation (A) and foliar δ^{13}C (B) for C$_3$ plants only. Points represent means $\pm 1\sigma$ for sites with $\geq$5 C$_3$ plant species sampled. Equation for δ^{15}N and MAP: $y = 8.8 - 1.1 \log x$, $r^2 = 0.49$; $p = 0.03$. Equation for δ^{13}C and MAP: $y = -30.1 + 0.5 \log x$, $r^2 = 0.81$; $p < 0.001$.
doi:10.1371/journal.pone.0053763.g011

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.
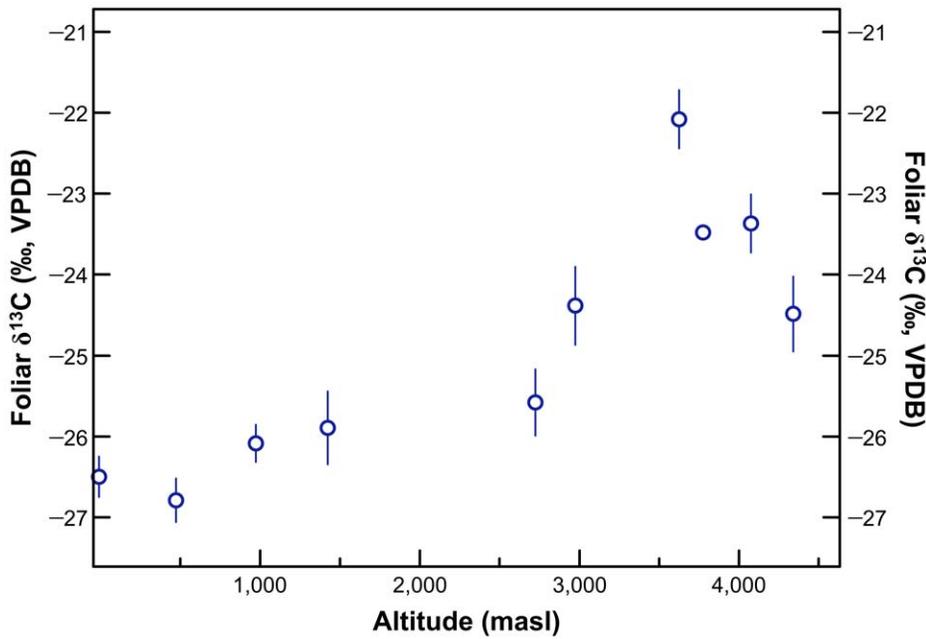
Figure 12. Bivariate plot of altitude and foliar δ^{13}C for plants collected in northern Chile [14].
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Wild Plants

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 N_2-fixation among leguminous trees in an arid region of southern Africa, with trees growing at the most arid sites showing no evidence of N_2-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 N_2-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 NO_3^- 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

Table 10. Mean ($\pm1\sigma$) isotopic and elemental compositions for marine algae.

Taxonomic Name	Type	n	δ^{13}C (‰, VPDB)	δ^{15}N (‰, 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 chamissoi	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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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 ≤1 ‰, but was absent for C_4 plants. This fits with previously described data for other plants. The small difference in δ^{13}C among plant tissues is not likely to be significant with respect to the interpretation of isotopic data in the context of paleodietary studies.

Geographic Variation in Carbon and Nitrogen Isotopic Compositions. There were strong relationships between sampling site and foliar carbon and nitrogen isotopic compositions. Foliar δ^{15}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 δ^{15}N [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 δ^{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 grain δ^{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 δ^{15}N values would be expected to be more limited (Figure 10a).

The positive relationship found between rainfall and foliar δ^{13}C in C_3 plants contrasts with most other studies, which have typically found a negative relationship between rainfall and foliar δ^{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 δ^{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 high carboxylation rates relative to stomatal conductance at high altitudes resulting in 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 cultigens 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 very 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 camelids (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 δ^{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 δ^{13}C values are more difficult to evaluate. While the positive linear relationship between altitude and foliar δ^{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 δ^{13}C value of available forage would still be higher at low altitude sites. Thus, markedly higher δ^{13}C and δ^{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 δ^{13}C values intermediate between C_3 and C_4 plants, with δ^{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 δ^{13}C and δ^{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 bird and/or mammalian excreta [217], there is no reason to expect marine algal δ^{15}N values to be higher than the δ^{15}N values of plants growing along the arid coast of Peru.

Conclusions

Maize from the study area has a mean δ^{13}C value of −11.8±0.4‰, which suggests that a δ^{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 δ^{15}N values and higher N contents than non-leguminous cultigens; this distinction was not as clear for wild legumes. Marine plants were characterized by δ^{13}C values intermediate between wild terrestrial C_3 and C_4 vegetation and δ^{15}N values that were very similar to terrestrial plants growing at low altitudes. C_4 plants were generally more abundant at lower altitude sites. Carbon and nitrogen isotopic compositions of wild plants were strongly influenced by local environmental factors. Foliar δ^{13}C was positively correlated with altitude and negatively correlated with mean annual precipitation. Foliar δ^{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)
(KML)

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

Author Contributions

Conceived and designed the experiments: PS CDW FJL JFM VFVS. Performed the experiments: PS. Analyzed the data: PS CDW FJL. Wrote the paper: PS.

References

1. DeNiro MJ, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. Geochimica et Cosmochimica Acta 42: 495–506.
2. DeNiro MJ, Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals. Geochimica et Cosmochimica Acta 45: 341–351.
3. Szpak P, Orchard TJ, McKechnie I, Gröcke DR (2012) Historical ecology of late Holocene sea otters (*Enhydra lutris*) from northern British Columbia: isotopic and zooarchaeological perspectives. Journal of Archaeological Science 39: 1553–1571.
4. Caut S, Angulo E, Courchamp F (2009) Variation in discrimination factors (Δ^{15}N and Δ^{13}C): the effect of diet isotopic values and applications for diet reconstruction. Journal of Applied Ecology 46: 443–453.
5. Casey MM, Post DM (2011) The problem of isotopic baseline: Reconstructing the diet and trophic position of fossil animals. Earth-Science Reviews 106: 131–148.
6. Hobson KA (1999) Tracing origins and migration of wildlife using stable isotopes: a review. Oecologia 120: 314–326.
7. Katzenberg MA, Weber A (1999) Stable Isotope Ecology and Palaeodiet in the Lake Baikal Region of Siberia. Journal of Archaeological Science 26: 651–659.
8. Szpak P, Orchard TJ, Gröcke DR (2009) A Late Holocene vertebrate food web from southern Haida Gwaii (Queen Charlotte Islands, British Columbia). Journal of Archaeological Science 36: 2734–2741.
9. Bösl C, Grupe G, Peters J (2006) A Late Neolithic vertebrate food web based on stable isotope analyses. International Journal of Osteoarchaeology 16: 296–315.
10. Grupe G, Heinrich D, Peters J (2009) A brackish water aquatic foodweb: trophic levels and salinity gradients in the Schlei fjord, Northern Germany, in Viking and medieval times. Journal of Archaeological Science 36: 2125–2144.
11. Kohn MJ (2010) Carbon isotope compositions of terrestrial C3 plants as indicators of (paleo)ecology and (paleo)climate. Proceedings of the National Academy of Sciences 107: 19691–19695.
12. Craine JM, Elmore AJ, Aidar MPM, Bustamante M, Dawson TE, et al. (2009) Global patterns of foliar nitrogen isotopes and their relationships with climate, mycorrhizal fungi, foliar nutrient concentrations, and nitrogen availability. New Phytologist 183: 980–992.
13. Codron J, Codron D, Lee-Thorp JA, Sponheimer M, Bond WJ, et al. (2005) Taxonomic, anatomical, and spatio-temporal variations in the stable carbon and nitrogen isotopic compositions of plants from an African savanna. Journal of Archaeological Science 32: 1757–1772.
14. Tieszen LL, Chapman M (1992) Carbon and nitrogen isotopic status of the major marine and terrestrial resources in the Atacama Desert of northern Chile. Proceedings of the First World Congress on Mummy Studies. Santa Cruz de Tenerife: Museo Arqueológico y Etnográfico de Tenerife. 409–425.
15. Hartman G, Danin A (2010) Isotopic values of plants in relation to water availability in the Eastern Mediterranean region. Oecologia 162: 837–852.
16. Ehleringer JR, Rundel PW, Palma B, Mooney HA (1998) Carbon isotope ratios of Atacama Desert plants reflect hyperaridity of region in northern Chile. Revista Chilena de Historia Natural 71: 79–86.
17. Panarello HO, Fernández J (2002) Stable carbon isotope measurements on hair from wild animals from altiplanic environments of Jujuy, Argentina. Radiocarbon 44: 709–716.
18. Gil AF, Neme GA, Tykot RH, Novellino P, Cortegoso V, et al. (2009) Stable isotopes and maize consumption in central western Argentina. International Journal of Osteoarchaeology 19: 215–236.
19. Izeta AD, Laguens AG, Marconetto MB, Scattolin MC (2009) Camelid handling in the meridional Andes during the first millennium AD: a preliminary approach using stable isotopes. International Journal of Osteoarchaeology 19: 204–214.
20. Gil AF, Tykot RH, Neme G, Shelnut NR (2006) Maize on the Frontier: Isotopic and Macrobotanical Data from Central-Western Argentina. In: Staller JE, Tykot RH, Benz BF, editors. Histories of Maize. Amsterdam: Elsevier Academic Press. pp. 199–214.
21. Martínez G, Zangrando AF, Prates L (2009) Isotopic ecology and human palaeodiets in the lower basin of the Colorado River, Buenos Aires province, Argentina. International Journal of Osteoarchaeology 19: 281–296.
22. Falabella F, Planella MT, Aspillaga E (2007) Dieta en sociedades alfareras de Chile Central: Aporte de análisis de isótopos estables. Chungará (Arica) 39: 5–27.
23. Rundel PW, Gibson AC, Midgley GS, Wand SJE, Palma B, et al. (2002) Ecological and ecophysiological patterns in a pre-altiplano shrubland of the Andean Cordillera in northern Chile. Plant Ecology 169: 179–193.
24. Latorre C, González AL, Quade J, Fariña JM, Pinto R, et al. (2011) Establishment and formation of fog-dependent *Tillandsia landbeckii* dunes in the Atacama Desert: Evidence from radiocarbon and stable isotopes. Journal of Geophysical Research 116: G03033.
25. Miller MJ, Capriles JM, Hastorf CA (2010) The fish of Lake Titicaca: implications for archaeology and changing ecology through stable isotope analysis. Journal of Archaeological Science 37: 317–327.
26. Ehleringer JR, Cooper DA, Lott MJ, Cook CS (1999) Geo-location of heroin and cocaine by stable isotope ratios. Forensic Science International 106: 27–35.
27. DeNiro MJ, Hastorf CA (1985) Alteration of ^{15}N/^{14}N and ^{13}C/^{12}C ratios of plant matter during the initial stages of diagenesis: studies utilizing archaeological specimens from Peru. Geochimica et Cosmochimica Acta 49: 97–115.
28. Catenazzi A, Donnelly MA (2007) Distribution of geckos in northern Peru: Long-term effect of strong ENSO events? Journal of Arid Environments 71: 327–332.
29. Townsend-Small A, McClain ME, Brandes JA (2005) Contributions of Carbon and Nitrogen from the Andes Mountains to the Amazon River: Evidence from an Elevational Gradient of Soils, Plants, and River Material. Limnology and Oceanography 50: 672–685.
30. Turner BL, Kingston JD, Armelagos GJ (2010) Variation in dietary histories among the immigrants of Machu Picchu: Carbon and nitrogen isotope evidence. Revista Chungara. Revista de Antropologia Chilena 42: 515–524.
31. Finucane B, Agurto PM, Isbell WH (2006) Human and animal diet at Conchopata, Peru: stable isotope evidence for maize agriculture and animal management practices during the Middle Horizon. Journal of Archaeological Science 33: 1766–1776.
32. Finucane BC (2007) Mummies, maize, and manure: multi-tissue stable isotope analysis of late prehistoric human remains from the Ayacucho Valley, Peru. Journal of Archaeological Science 34: 2115–2124.
33. Finucane BC (2008) Trophy heads from Nawinpukio, Peru: Physical and chemical analysis of Huarpa-era modified human remains. American Journal of Physical Anthropology 135: 75–84.
34. Finucane BC (2009) Maize and Sociopolitical Complexity in the Ayacucho Valley, Peru. Current Anthropology 50: 535–545.
35. Kellner CM, Schoeninger MJ (2008) Wari's imperial influence on local Nasca diet: The stable isotope evidence. Journal of Anthropological Archaeology 27: 226–243.
36. Knudson KJ, Aufderheide AE, Buikstra JE (2007) Seasonality and paleodiet in the Chiribaya polity of southern Peru. Journal of Archaeological Science 34: 451–462.
37. Slovak NM, Paytan A (2011) Fisherfolk and farmers: Carbon and nitrogen isotope evidence from Middle Horizon Ancón, Peru. International Journal of Osteoarchaeology 21: 253–267.
38. Thornton EK, Defrance SD, Krigbaum J, Williams PR (2011) Isotopic evidence for Middle Horizon to 16th century camelid herding in the Osmore Valley, Peru. International Journal of Osteoarchaeology 21: 544–567.
39. Tomczak PD (2003) Prehistoric diet and socioeconomic relationships within the Osmore Valley of southern Peru. Journal of Anthropological Archaeology 22: 262–278.
40. White CD, Nelson AJ, Longstaffe FJ, Grupe G, Jung A (2009) Landscape bioarchaeology at Pacatnamu, Peru: inferring mobility from δ^{13}C and δ^{15}N values of hair. Journal of Archaeological Science 36: 1527–1537.
41. Williams JS, Katzenberg MA (2012) Seasonal fluctuations in diet and death during the late horizon: a stable isotopic analysis of hair and nail from the central coast of Peru. Journal of Archaeological Science 39: 41–57.
42. Tykot RH, Burger RL, van der Merwe NJ (2006) The Importance of Maize in Initial Period and Early Horizon Peru. In: Staller JE, Tykot RH, Benz BF, editors. Histories of Maize. Amsterdam: Elsevier Academic Press. pp. 187–197.
43. Berón MA, Luna LH, Barberena R (2009) Isotopic archaeology in the western Pampas (Argentina): preliminary results and perspectives. International Journal of Osteoarchaeology 19: 250–265.

44. Politis GG, Scabuzzo C, Tykot RH (2009) An approach to pre-Hispanic diets in the Pampas during the Early/Middle Holocene. International Journal of Osteoarchaeology 19: 266–280.

45. Tessone A, Zangrando AF, Barrientos G, Goñi R, Panarello H, et al. (2009) Stable isotope studies in the Salitroso Lake Basin (southern Patagonia, Argentina): assessing diet of Late Holocene hunter-gatherers. International Journal of Osteoarchaeology 19: 297–308.

46. Yacobaccio HD, Morales MR, Samec CT (2009) Towards an isotopic ecology of herbivory in the Puna ecosystem: new results and patterns on *Lama glama*. International Journal of Osteoarchaeology 19: 144–155.

47. Gil AF, Neme GA, Tykot RH (2011) Stable isotopes and human diet in central western Argentina. Journal of Archaeological Science 38: 1395–1404.

48. Ericson JE, West M, Sullivan CH, Krueger HW (1989) The Development of Maize Agriculture in the Viru Valley, Peru. In: Price TD, editor. The Chemistry of Prehistoric Human Bone. Cambridge: Cambridge University Press. pp. 68–104.

49. Verano JW, DeNiro MJ (1993) Locals or foreigners? Morphological, biometric and isotopic approaches to the question of group affinity in human skeletal remains recovered from unusual archaeological context. In: Sandford MK, editor. Investigations of Ancient Human Tissue: Chemical Analysis in Anthropology. Langhorne: Gordon and Breach. pp. 361–386.

50. Bush MB, Hansen BCS, Rodbell DT, Seltzer GO, Young KR, et al. (2005) A 17 000-year history of Andean climate and vegetation change from Laguna de Chochos, Peru. Journal of Quaternary Science 20: 703–714.

51. Brush SB (1982) The Natural and Human Environment of the Central Andes. Mountain Research and Development 2: 19–38.

52. Sandweiss DH, Richardson JB, III (2008) Central Andean environments. In: Silverman H, Isbell WH, editors. Handbook of South American Archaeology. New York: Springer. pp. 93–104.

53. Troll C (1968) The cordilleras of the tropical Americas. In: Troll C, editor. Geoecology of the Mountainous Regions of the Tropical Americas. Proceedings of the UNESCO Mexico Symposium Colloquium Geographicum Volume 9. Bonn: Geographisches Institut der Universtat. pp. 15–56.

54. Tosi JA, Jr. (1960) Zonas de vida natural en el Peru. Lima, Peru: Instituto de Ciencias Agrícolas de la OEA, Zona Andina.

55. Rundel PW, Dillon MO, Palma B, Mooney HA, Gulmon SL (1991) The phytogeography and ecology of the coastal Atacama and Peruvian deserts. Aliso 13: 1–49.

56. Winterhalder BP, Thomas RB (1978) Geoecology of southern highland Peru. Boulder: Occasional Paper of the University of Colorado, Institute of Arctic and Alpine Research No.27.

57. Koepcke H-W (1961) Synökologische Studien an der Westseite der Peruanischen Anden. Bonn: Donner Geographische Abhandlungen Heft 29.

58. Koepcke M (1954) Corte ecológica transversal en los Andes del Perú Central con especial consideración de los aves. Lima, Peru: Universidad Nacional Mayor de San Marcos, Museo de Historia Natural "Javier Pradom" Memorias no.3.

59. de Mera AG, Orellana JAV, Garcia JAL (1997) Phytogeographical Sectoring of the Peruvian Coast. Global Ecology and Biogeography Letters 6: 349–367.

60. Ono M, editor (1986) Taxonomic and Ecological Studies on the Lomas Vegetation in the Pacific Coast of Peru. Tokyo: Makino Herbarium, Tokyo Metropolitan University. 88 p.

61. Péfaur JE (1982) Dynamics of plant communities in the Lomas of southern Peru. Plant Ecology 49: 163–171.

62. Oka S, Ogawa H (1984) The distribution of lomas vegetation and its climatic environments along the Pacific coast of Peru. In: Matsuda I, Harayama M, Suzuki K, editors. Geographical Reports of the Tokyo Metropolitan University, Number 19. Tokyo: Department of Geography, Tokyo Metropolitan University. pp. 113–125.

63. West M (1971) Prehistoric Human Ecology in the Virú Valley. California Anthropologist 1: 47–56.

64. Beresford-Jones DG, T SA, Whaley OQ, Chepstow-Lusty AJ (2009) The role of *Prosopis* in ecological and landscape change in the Samaca Basin, Lower Ica Valley, South Coast Peru from the Early Horizon to the Late Intermediate Period. Latin American Antiquity 20: 303–332.

65. Smith BN, Epstein S (1971) Two Categories of $^{13}C/^{12}C$ Ratios for Higher Plants. Plant Physiology 47: 380–384.

66. O'Leary MH (1981) Carbon isotope fractionation in plants. Phytochemistry 20: 553–567.

67. Schwarcz HP (2006) Stable Carbon Isotope Analysis and Human Diet. In: Staller JE, Tykot RH, Benz BF, editors. Histories of Maize. Amsterdam: Elsevier Academic Press. pp. 315–321.

68. O'Leary MH (1988) Carbon Isotopes in Photosynthesis. Bioscience 38: 328–336.

69. Rice SK (2000) Variation in carbon isotope discrimination within and among *Sphagnum* species in a temperate wetland. Oecologia 123: 1–8.

70. Rundel PW, Stichler W, Zander RH, Ziegler H (1979) Carbon and hydrogen isotope ratios of bryophytes from arid and humid regions. Oecologia 44: 91–94.

71. Lange O, Green T, Ziegler H (1988) Water status related photosynthesis and carbon isotope discrimination in species of the lichen genus *Pseudocyphellaria* with green or blue-green photobionts and in photosymbiodemes. Oecologia 75: 494–501.

72. Lee Y, Lim H, Yoon H (2009) Carbon and nitrogen isotope composition of vegetation on King George Island, maritime Antarctic. Polar Biology 32: 1607–1615.

73. Huiskes A, Boschker H, Lud D, Moerdijk-Poortvliet T (2006) Stable Isotope Ratios as a Tool for Assessing Changes in Carbon and Nutrient Sources in Antarctic Terrestrial Ecosystems. Plant Ecology 182: 79–86.

74. van der Merwe NJ, Medina E (1989) Photosynthesis and $^{13}C/^{12}C$ ratios in Amazonian rain forests. Geochimica et Cosmochimica Acta 53: 1091–1094.

75. van der Merwe NJ, Medina E (1991) The canopy effect, carbon isotope ratios and foodwebs in Amazonia. Journal of Archaeological Science 18: 249–259.

76. Sonesson M, Gehrke C, Tjus M (1992) CO_2 environment, microclimate and photosynthetic characteristics of the moss *Hylocomium splendens* in a subarctic habitat. Oecologia 92: 23–29.

77. Medina E, Sternberg L, Cuevas E (1991) Vertical stratification of $\delta^{13}C$ values in closed natural and plantation forests in the Luquillo mountains, Puerto Rico. Oecologia 87: 369–372.

78. Vogel JC (1978) Recycling of CO_2 in a forest environment. Oecologia Plantarum 13: 89–94.

79. Buchmann N, Guehl JM, Barigah TS, Ehleringer JR (1997) Interseasonal comparison of CO_2 concentrations, isotopic composition, and carbon dynamics in an Amazonian rainforest (French Guiana). Oecologia 110: 120–131.

80. Broadmeadow MSJ, Griffiths H (1993) Carbon isotope discrimination and the coupling of CO_2 fluxes within forest canopies. In: Ehleringer JR, Hall AE, Farquhar GD, editors. Stable Isotopes and Plant Carbon-Water Relations. San Diego: Academic Press. pp. 109–129.

81. Heaton THE (1999) Spatial, species, and temporal variations in the $^{13}C/^{12}C$ ratios of C_3 plants: implications for palaeodiet studies. Journal of Archaeological Science 26: 637–649.

82. Drucker DG, Bridault A, Hobson KA, Szuma E, Bocherens H (2008) Can carbon-13 in large herbivores reflect the canopy effect in temperate and boreal ecosystems? Evidence from modern and ancient ungulates. Palaeogeography, Palaeoclimatology, Palaeoecology 266: 69–82.

83. Voigt CC (2010) Insights into Strata Use of Forest Animals Using the 'Canopy Effect'. Biotropica 42: 634–637.

84. Schoeninger MJ, Iwaniec UT, Glander KE (1997) Stable isotope ratios indicate diet and habitat use in New World monkeys. American Journal of Physical Anthropology 103: 69–83.

85. Austin AT, Vitousek PM (1998) Nutrient dynamics on a precipitation gradient in Hawai'i. Oecologia 113: 519–529.

86. Swap RJ, Aranibar JN, Dowty PR, Gilhooly WP, III, Macko SA (2004) Natural abundance of ^{13}C and ^{15}N in C_3 and C_4 vegetation of southern Africa: patterns and implications. Global Change Biology 10: 350–358.

87. Murphy BP, Bowman DMJS (2009) The carbon and nitrogen isotope composition of Australian grasses in relation to climate. Functional Ecology 23: 1040–1049.

88. Lajtha K, Getz J (1993) Photosynthesis and water-use efficiency in pinyon-juniper communities along an elevation gradient in northern New Mexico. Oecologia 94: 95–101.

89. Scartazza A, Lauteri M, Guido MC, Brugnoli E (1998) Carbon isotope discrimination in leaf and stem sugars, water-use efficiency and mesophyll conductance during different developmental stages in rice subjected to drought. Australian Journal of Plant Physiology 25: 489–498.

90. Syvertsen JP, Smith ML, Lloyd J, Farquhar GD (1997) Net Carbon Dioxide Assimilation, Carbon Isotope Discrimination, Growth, and Water-use Efficiency of Citrus Trees in Response to Nitrogen Status. Journal of the American Society for Horticultural Science 122: 226–232.

91. Damesin C, Rambal S, Joffre R (1997) Between-tree variations in leaf $\delta^{13}C$ of *Quercus pubescens* and *Quercus ilex* among Mediterranean habitats with different water availability. Oecologia 111: 26–35.

92. Schulze ED, Ellis R, Schulze W, Trimborn P, Ziegler H (1996) Diversity, metabolic types and $\delta^{13}C$ carbon isotope ratios in the grass flora of Namibia in relation to growth form, precipitation and habitat conditions. Oecologia 106: 352–369.

93. Farquhar G, Richards R (1984) Isotopic Composition of Plant Carbon Correlates With Water-Use Efficiency of Wheat Genotypes. Australian Journal of Plant Physiology 11: 539–552.

94. Farquhar G, O'Leary M, Berry J (1982) On the Relationship Between Carbon Isotope Discrimination and the Intercellular Carbon Dioxide Concentration in Leaves. Australian Journal of Plant Physiology 9: 121–137.

95. Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon Isotope Discrimination and Photosynthesis. Annual Review of Plant Physiology and Plant Molecular Biology 40: 503–537.

96. Guy RD, Reid DM, Krouse HR (1980) Shifts in carbon isotope ratios of two C_3 halophytes under natural and artificial conditions. Oecologia 44: 241–247.

97. Farquhar GD, Ball MC, Caemmerer S, Roksandic Z (1982) Effect of salinity and humidity on $\delta^{13}C$ value of halophytes–Evidence for diffusional isotope fractionation determined by the ratio of intercellular/atmospheric partial pressure of CO_2 under different environmental conditions. Oecologia 52: 121–124.

98. van Groenigen J-W, van Kessel C (2002) Salinity-induced Patterns Of Natural Abundance Carbon-13 And Nitrogen-15 In Plant And Soil. Soil Science Society of America Journal 66: 489–498.

99. Isla R, Aragüés R, Royo A (1998) Validity of various physiological traits as screening criteria for salt tolerance in barley. Field Crops Research 58: 97–107.

100. Vitousek PM, Field CB, Matson PA (1990) Variation in foliar δ¹³C in Hawaiian *Metrosideros polymorpha*: a case of internal resistance? Oecologia 84: 362–370.

101. Hultine KR, Marshall JD (2000) Altitude trends in conifer leaf morphology and stable carbon isotope composition. Oecologia 123: 32–40.

102. Körner C, Diemer M (1987) In situ Photosynthetic Responses to Light, Temperature and Carbon Dioxide in Herbaceous Plants from Low and High Altitude. Functional Ecology 1: 179–194.

103. Friend AD, Woodward FI, Switsur VR (1989) Field Measurements of Photosynthesis, Stomatal Conductance, Leaf Nitrogen and δ¹³C Along Altitudinal Gradients in Scotland. Functional Ecology 3: 117–122.

104. Körner C, Farquhar GD, Wong SC (1991) Carbon isotope discrimination by plants follows latitudinal and altitudinal trends. Oecologia 88: 30–40.

105. Ehleringer JR, Field CB, Lin Z-f, Kuo C-y (1986) Leaf carbon isotope and mineral composition in subtropical plants along an irradiance cline. Oecologia 70: 520–526.

106. Zimmerman J, Ehleringer J (1990) Carbon isotope ratios are correlated with irradiance levels in the Panamanian orchid *Catasetum viridiflavum*. Oecologia 83: 247–249.

107. Badeck F-W, Tcherkez G, Nogués S, Piel C, Ghashghaie J (2005) Post-photosynthetic fractionation of stable carbon isotopes between plant organs–a widespread phenomenon. Rapid Communications in Mass Spectrometry 19: 1381–1391.

108. Hobbie EA, Werner RA (2004) Intramolecular, compound-specific, and bulk carbon isotope patterns in C₃ and C₄ plants: a review and synthesis. New Phytologist 161: 371–385.

109. Cernusak LA, Tcherkez G, Keitel C, Cornwell WK, Santiago LS, et al. (2009) Why are non-photosynthetic tissues generally ¹³C enriched compared with leaves in C₃ plants? Review and synthesis of current hypotheses. Functional Plant Biology 36: 199–213.

110. Damesin C, Lelarge C (2003) Carbon isotope composition of current-year shoots from *Fagus sylvatica* in relation to growth, respiration and use of reserves. Plant, Cell & Environment 26: 207–219.

111. Leavitt SW, Long A (1982) Evidence for ¹³C/¹²C fractionation between tree leaves and wood. Nature 298: 742–744.

112. DeNiro MJ, Epstein S (1977) Mechanism of Carbon Isotope Fractionation Associated with Lipid Synthesis. Science 197: 261–263.

113. Benner R, Fogel ML, Sprague EK, Hodson RE (1987) Depletion of ¹³C in lignin and its implications for stable carbon isotope studies. Nature 329: 708–710.

114. Gleixner G, Danier HJ, Werner RA, Schmidt HL (1993) Correlations between the ¹³C Content of Primary and Secondary Plant Products in Different Cell Compartments and That in Decomposing Basidiomycetes. Plant Physiology 102: 1287–1290.

115. Terwilliger VJ, Huang J (1996) Heterotrophic whole plant tissues show more 13C enrichment than their carbon sources. Phytochemistry 43: 1183–1188.

116. Ambrose SH, Butler BM, Hanson DB, Hunter-Anderson RL, Krueger HW (1997) Stable isotopic analysis of human diet in the Marianas Archipelago, Western Pacific. American Journal of Physical Anthropology 104: 343–361.

117. Schoeninger MJ, DeNiro MJ (1984) Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. Geochimica et Cosmochimica Acta 48: 625–639.

118. Xu J, Fan X, Zhang X, Xu D, Mou S, et al. (2012) Evidence of Coexistence of C₃ and C₄ Photosynthetic Pathways in a Green-Tide-Forming Alga, *Ulva prolifera*. PLoS One 7: e37438.

119. Raven JA, Johnston AM, Kübler JE, Korb R, McInroy SG, et al. (2002) Mechanistic interpretation of carbon isotope discrimination by marine macroalgae and seagrasses. Functional Plant Biology 29: 355–378.

120. Kroopnick PM (1985) The distribution of ¹³C of ΣCO₂ in the world oceans. Deep Sea Research Part A. Oceanographic Research Papers 32: 57–84.

121. France RL (1995) Carbon-13 enrichment in benthic compared to planktonic algae: foodweb implications. Marine Ecology Progress Series 124: 307–312.

122. Osmond CB, Valaane N, Haslam SM, Uotila P, Roksandic Z (1981) Comparisons of δ¹³C values in leaves of aquatic macrophytes from different habitats in Britain and Finland; some implications for photosynthetic processes in aquatic plants. Oecologia 50: 117–124.

123. Cornelisen CD, Wing SR, Clark KL, Bowman MH, Frew RD, et al. (2007) Patterns in the δ¹³C and δ¹⁵N signature of *Ulva pertusa*: Interaction between physical gradients and nutrient source pools. Limnology and Oceanography 52: 820–832.

124. Burkhardt S, Riebesell U, Zondervan I (1999) Effects of growth rate, CO₂ concentration, and cell size on the stable carbon isotope fractionation in marine phytoplankton. Geochimica et Cosmochimica Acta 63: 3729–3741.

125. Kopczyńska EE, Goeyens L, Semeneh M, Dehairs F (1995) Phytoplankton composition and cell carbon distribution in Prydz Bay, Antarctica: relation to organic particulate matter and its δ¹³C values. Journal of Plankton Research 17: 685–707.

126. Laws EA, Popp BN, Bidigare RR, Kennicutt MC, Macko SA (1995) Dependence of phytoplankton carbon isotopic composition on growth rate and [CO₂]ₐq: Theoretical considerations and experimental results. Geochimica et Cosmochimica Acta 59: 1131–1138.

127. Wiencke C, Fischer G (1990) Growth and stable carbon isotope composition of cold-water macroalgae in relation to light and temperature. Marine Ecology Progress Series 65: 283–292.

128. Miller R, Page H (2012) Kelp as a trophic resource for marine suspension feeders: a review of isotope-based evidence. Marine Biology 159: 1391–1402.

129. Pilbeam DJ (2010) The Utilization of Nitrogen by Plants: A Whole Plant Perspective. Annual Plant Reviews 42: 305–351.

130. Yoneyama T, Ito O, Engelaar WMHG (2003) Uptake, metabolism and distribution of nitrogen in crop plants traced by enriched and natural ¹⁵N: Progress over the last 30 years. Phytochemistry Reviews 2: 121–132.

131. Vitousek PM, Cassman K, Cleveland C, Crews T, Field CB, et al. (2002) Towards an ecological understanding of biological nitrogen fixation. Biogeochemistry 57–58: 1–45.

132. Persson J, Näsholm T (2001) Amino acid uptake: a widespread ability among boreal forest plants. Ecology Letters 4: 434–438.

133. Jones DL, Healey JR, Willett VB, Farrar JF, Hodge A (2005) Dissolved organic nitrogen uptake by plants–an important N uptake pathway? Soil Biology and Biochemistry 37: 413–423.

134. Näsholm T, Kielland K, Ganeteg U (2009) Uptake of organic nitrogen by plants. New Phytologist 182: 31–48.

135. Högberg P (1997) Tansley Review No. 95 ¹⁵N natural abundance in soil-plant systems. New Phytologist 137: 179–203.

136. Evans RD (2001) Physiological mechanisms influencing plant nitrogen isotope composition. Trends in Plant Science 6: 121–126.

137. Robinson D (2001) δ¹⁵N as an integrator of the nitrogen cycle. Trends in Ecology & Evolution 16: 153–162.

138. Delwiche CC, Steyn PL (1970) Nitrogen isotope fractionation in soils and microbial reactions. Environmental Science & Technology 4: 929–935.

139. Shearer G, Kohl DH (1986) N₂-Fixation in Field Settings: Estimations Based on Natural ¹⁵N Abundance. Australian Journal of Plant Physiology 13: 699–756.

140. Mariotti A, Mariotti F, Amargar N, Pizelle G, Ngambi JM, et al. (1980) Fractionnements isotopiques de l'azote lors des processus d'absorption des nitrates et de fixation de l'azote atmosphérique par les plantes. Physiologie Végétale 18: 163–181.

141. Mariotti A (1983) Atmospheric nitrogen is a reliable standard for natural ¹⁵N abundance measurements. Nature 303: 685–687.

142. Shearer G, Kohl DH, Chien S-H (1978) The Nitrogen-15 Abundance In A Wide Variety Of Soils. Soil Science Society of America Journal 42: 899–902.

143. Szpak P, Longstaffe FJ, Millaire J-F, White CD (2012) Stable Isotope Biogeochemistry of Seabird Guano Fertilization: Results from Growth Chamber Studies with Maize (*Zea mays*). PLoS One 7: e33741.

144. Bateman AS, Kelly SD (2007) Fertilizer nitrogen isotope signatures. Isotopes in Environmental and Health Studies 43: 237–247.

145. Choi W-J, Lee S-M, Ro H-M, Kim K-C, Yoo S-H (2002) Natural ¹⁵N abundances of maize and soil amended with urea and composted pig manure. Plant and Soil 245: 223–232.

146. Yun S-I, Ro H-M, Choi W-J, Chang SX (2006) Interactive effects of N fertilizer source and timing of fertilization leave specific N isotopic signatures in Chinese cabbage and soil. Soil Biology and Biochemistry 38: 1682–1689.

147. Szpak P, Millaire J-F, White CD, Longstaffe FJ (2012) Influence of seabird guano and camelid dung fertilization on the nitrogen isotopic composition of field-grown maize (*Zea mays*). Journal of Archaeological Science 39: 3721–3740.

148. Ben-David M, Hanley TA, Schell DM (1998) Fertilization of Terrestrial Vegetation by Spawning Pacific Salmon: The Role of Flooding and Predator Activity. Oikos 83: 47–55.

149. Hilderbrand GV, Hanley TA, Robbins CT, Schwartz CC (1999) Role of brown bears (*Ursus arctos*) in the flow of marine nitrogen into a terrestrial ecosystem. Oecologia 121: 546–550.

150. Li C, Hao X, Willms WD, Zhao M, Han G (2010) Effect of long-term cattle grazing on seasonal nitrogen and phosphorus concentrations in range forage species in the fescue grassland of southwestern Alberta. Journal of Plant Nutrition and Soil Science 173: 946–951.

151. Coetsee C, Stock WD, Craine JM (2011) Do grazers alter nitrogen dynamics on grazing lawns in a South African savannah? African Journal of Ecology 49: 62–69.

152. Golluscio R, Austin A, García Martínez G, Gonzalez-Polo M, Sala O, et al. (2009) Sheep Grazing Decreases Organic Carbon and Nitrogen Pools in the Patagonian Steppe: Combination of Direct and Indirect Effects. Ecosystems 12: 686–697.

153. Frank DA, Evans RD (1997) Effects of native grazers on grassland in cycling in Yellowstone National Park. Ecology 78: 2238–2248.

154. Wittmer M, Auerswald K, Schönbach P, Bai Y, Schnyder H (2011) ¹⁵N fractionation between vegetation, soil, faeces and wool is not influenced by stocking rate. Plant and Soil 340: 25–33.

155. Xu Y, He J, Cheng W, Xing X, Li L (2010) Natural ¹⁵N abundance in soils and plants in relation to N cycling in a rangeland in Inner Mongolia. Journal of Plant Ecology 3: 201–207.

156. Frank D, Evans RD, Tracy B (2004) The role of ammonia volatilization in controlling the natural ¹⁵N abundance of a grazed grassland. Biogeochemistry 68: 169–178.

157. Högberg P (1990) 15N natural abundance as a possible marker of the ectomycorrhizal habit of trees in mixed African woodlands. New Phytologist 115: 483–486.

158. Michelsen A, Quarmby C, Sleep D, Jonasson S (1998) Vascular plant ¹⁵N natural abundance in heath and forest tundra ecosystems is closely correlated with presence and type of mycorrhizal fungi in roots. Oecologia 115: 406–418.

159. Högberg P, Högbom L, Schinkel H, Högberg M, Johannisson C, et al. (1996) ^{15}N abundance of surface soils, roots and mycorrhizas in profiles of European forest soils. Oecologia 108: 207–214.

160. Michelsen A, Schmidt IK, Jonasson S, Quarmby C, Sleep D (1996) Leaf ^{15}N abundance of subarctic plants provides field evidence that ericoid, ectomycorrhizal and non-and arbuscular mycorrhizal species access different sources of soil nitrogen. Oecologia 105: 53–63.

161. Schulze ED, Chapin FS, Gebauer G (1994) Nitrogen nutrition and isotope differences among life forms at the northern treeline of Alaska. Oecologia 100: 406–412.

162. Hobbie E, Jumpponen A, Trappe J (2005) Foliar and fungal ^{15}N:^{14}N ratios reflect development of mycorrhizae and nitrogen supply during primary succession: testing analytical models. Oecologia 146: 258–268.

163. Hobbie EA, Macko SA, Shugart HH (1999) Insights into nitrogen and carbon dynamics of ectomycorrhizal and saprotrophic fungi from isotopic evidence. Oecologia 118: 353–360.

164. Hobbie EA, Colpaert JV (2003) Nitrogen availability and colonization by mycorrhizal fungi correlate with nitrogen isotope patterns in plants. New Phytologist 157: 115–126.

165. Ledgard SF, Woo KC, Bergersen FJ (1985) Isotopic fractionation during reduction of nitrate and nitrite by extracts of spinach leaves. Australian Journal of Plant Physiology 12: 631–640.

166. Evans RD, Bloom AJ, Sukrapanna SS, Ehleringer JR (1996) Nitrogen isotope composition of tomato (Lycopersicon esculentum Mill. cv. T-5) grown under ammonium or nitrate nutrition. Plant, Cell & Environment 19: 1317–1323.

167. Crawford TW, Rendig VV, Broadbent FE (1982) Sources, Fluxes, and Sinks of Nitrogen during Early Reproductive Growth of Maize (Zea mays L.). Plant Physiology 70: 1654–1660.

168. Bada JL, Schoeninger MJ, Schimmelmann A (1989) Isotopic fractionation during peptide bond hydrolysis. Geochimica et Cosmochimica Acta 53: 3337–3341.

169. Silfer JA, Engel MH, Macko SA (1992) Kinetic fractionation of stable carbon and nitrogen isotopes during peptide bond hydrolysis: Experimental evidence and geochemical implications. Chemical Geology 101: 211–221.

170. Szpak P, Gröcke DR, Debruyne R, MacPhee RDE, Guthrie RD, et al. (2010) Regional differences in bone collagen δ^{13}C and δ^{15}N of Pleistocene mammoths: Implications for paleoecology of the mammoth steppe. Palaeogeography, Palaeoclimatology, Palaeoecology 286: 88–96.

171. Gröcke DR, Bocherens H, Mariotti A (1997) Annual rainfall and nitrogen-isotope correlation in macropod collagen: application as a palaeoprecipitation indicator. Earth and Planetary Science Letters 153: 279–285.

172. Murphy BP, Bowman DMJS (2006) Kangaroo metabolism does not cause the relationship between bone collagen δ^{15}N and water availability. Functional Ecology 20: 1062–1069.

173. Sealy JC, van der Merwe NJ, Lee-Thorp JA, Lanham JL (1987) Nitrogen isotopic ecology in southern Africa: implications for environmental and dietary tracing. Geochimica et Cosmochimica Acta 51: 2707–2717.

174. Ambrose SH, DeNiro MJ (1986) The isotopic ecology of east African mammals. Oecologia 69: 395–406.

175. Schwarcz HP, Dupras TL, Fairgrieve SI (1999) ^{15}N enrichment in the Sahara: in search of a global relationship. Journal of Archaeological Science 26: 629–636.

176. Amundson R, Austin AT, Schuur EAG, Yoo K, Matzek V, et al. (2003) Global patterns of the isotopic composition of soil and plant nitrogen. Global Biogeochemical Cycles 17: 1031.

177. Martinelli LA, Piccolo MC, Townsend AR, Vitousek PM, Cuevas E, et al. (1999) Nitrogen stable isotopic composition of leaves and soil: Tropical versus temperate forests. Biogeochemistry 46: 45–65.

178. Aranibar JN, Otter L, Macko SA, Feral CJW, Epstein HE, et al. (2004) Nitrogen cycling in the soil-plant system along a precipitation gradient in the Kalahari sands. Global Change Biology 10: 359–373.

179. Handley LL, Austin AT, Stewart GR, Robinson D, Scrimgeour CM, et al. (1999) The ^{15}N natural abundance (δ^{15}N) of ecosystem samples reflects measures of water availability. Australian Journal of Plant Physiology 26: 185–199.

180. Ambrose SH (2000) Controlled diet and climate experiments on nitrogen isotope ratios of rats. In: Ambrose SH, Katzenberg MA, editors. Biogeochemical Approaches to Paleodietary Analysis. New York: Kluwer Academic. 243–259.

181. Hartman G (2011) Are elevated δ^{15}N values in herbivores in hot and arid environments caused by diet or animal physiology? Functional Ecology 25: 122–131.

182. Ostrom NE, Macko SA, Deibel D, Thompson RJ (1997) Seasonal variation in the stable carbon and nitrogen isotope biogeochemistry of a coastal cold ocean environment. Geochimica et Cosmochimica Acta 61: 2929–2942.

183. Waser NAD, Harrison PJ, Nielsen B, Calvert SE, Turpin DH (1998) Nitrogen Isotope Fractionation During the Uptake and Assimilation of Nitrate, Nitrite, Ammonium, and Urea by a Marine Diatom. Limnology and Oceanography 43: 215–224.

184. Wu J, Calvert SE, Wong CS (1997) Nitrogen isotope variations in the subarctic northeast Pacific: relationships to nitrate utilization and trophic structure. Deep Sea Research Part I: Oceanographic Research Papers 44: 287–314.

185. Sage R, Pearcy R (2004) The Physiological Ecology of C$_4$ Photosynthesis. In: Leegood RC, Sharkey TD, von Caemmerer S, editors. Photosynthesis: Physiology and Metabolism. New York: Kluwer Academic. 497–532.

186. Tieszen LL, Fagre T (1993) Carbon Isotopic Variability in Modern and Archaeological Maize. Journal of Archaeological Science 20: 25–40.

187. Warinner C, Garcia NR, Tuross N (2012) Maize, beans and the floral isotopic diversity of highland Oaxaca, Mexico. Journal of Archaeological Science doi: 10.1016/j.jas.2012.07.003.

188. Keeling CD (1979) The Suess effect: ^{13}Carbon-^{14}Carbon interrelations. Environment International 2: 229–300.

189. Yakir D (2011) The paper trail of the ^{13}C of atmospheric CO$_2$ since the industrial revolution period. Environmental Research Letters 6: 034007.

190. Belane A, Dakora F (2010) Symbiotic N$_2$ fixation in 30 field-grown cowpea (Vigna unguiculata L. Walp.) genotypes in the Upper West Region of Ghana measured using ^{15}N natural abundance. Biology and Fertility of Soils 46: 191–198.

191. Sprent JI, Geoghegan IE, Whitty PW, James EK (1996) Natural abundance of ^{15}N and ^{13}C in nodulated legumes and other plants in the cerrado and neighbouring regions of Brazil Oecologia 105: 440–446.

192. Yoneyama T, Fujita K, Yoshida T, Matsumoto T, Kambayashi I, et al. (1986) Variation in Natural Abundance of ^{15}N among Plant Parts and in ^{15}N/^{14}N Fractionation during N$_2$ Fixation in the Legume-Rhizobia Symbiotic System. Plant and Cell Physiology 27: 791–799.

193. Spriggs AC, Stock WD, Dakora FD (2003) Influence of mycorrhizal associations on foliar δ^{15}N values of legume and non-legume shrubs and trees in the fynbos of South Africa: Implications for estimating N$_2$ fixation using the ^{15}N natural abundance method Plant and Soil 255: 495–502.

194. Yoneyama T, Muraoka T, Murakami T, Boonkerd N (1993) Natural abundance of ^{15}N in tropical plants with emphasis on tree legumes Plant and Soil 153: 295–304.

195. Shearer G, Kohl DH, Virginia RA, Bryan BA, Skeeters JL, et al. (1983) Estimates of N$_2$-fixation from variation in the natural abundance of ^{15}N in Sonoran desert ecosystems. Oecologia 56: 365–373.

196. Delwiche CC, Zinke PJ, Johnson CM, Virginia RA (1979) Nitrogen isotope distribution as a presumptive indicator of nitrogen fixation. Botanical Gazette 140: S65–S69.

197. Virginia RA, Delwiche CC (1982) Natural ^{15}N abundance of presumed N$_2$-fixing and non-N$_2$-fixing plants from selected ecosystems. Oecologia 54: 317–325.

198. Steele KW, Bonish PM, Daniel RM, O'Hara GW (1983) Effect of Rhizobial Strain and Host Plant on Nitrogen Isotopic Fractionation in Legumes. Plant Physiology 72: 1001–1004.

199. Kohl DH, Shearer G (1980) Isotopic Fractionation Associated With Symbiotic N$_2$ Fixation and Uptake of NO$_3^-$ by Plants. Plant Physiology 66: 51–56.

200. Gathumbi SM, Cadisch G, Giller KE (2002) ^{15}N natural abundance as a tool for assessing N$_2$-fixation of herbaceous, shrub and tree legumes in improved fallows. Soil Biology and Biochemistry 34: 1059–1071.

201. Lightfoot E, Stevens RE (2012) Stable isotope investigations of charred barley (Hordeum vulgare) and wheat (Triticum spelta) grains from Danebury Hillfort: implications for palaeodietary reconstructions. Journal of Archaeological Science doi: 10.1016/j.jas.2011.10.026.

202. Aguilera M, Araus JL, Voltas J, Rodríguez-Ariza MO, Molina F, et al. (2008) Stable carbon and nitrogen isotopes and quality traits of fossil cereal grains provide clues on sustainability at the beginnings of Mediterranean agriculture. Rapid Communications in Mass Spectrometry 22: 1653–1663.

203. Fiorentino G, Caracuta V, Casiello G, Longobardi F, Sacco A (2012) Studying ancient crop provenance: implications from δ^{13}C and δ^{15}N values of charred barley in a Middle Bronze Age silo at Ebla (NW Syria). Rapid Communications in Mass Spectrometry 26: 327–335.

204. DeNiro MJ (1985) Postmortem preservation and alteration of in vivo bone collagen isotope ratios in relation to palaeodietary reconstruction. Nature 317: 806–809.

205. Ambrose SH (1990) Preparation and characterization of bone and tooth collagen for isotopic analysis. Journal of Archaeological Science 17: 431–451.

206. van Klinken GJ (1999) Bone Collagen Quality Indicators for Palaeodietary and Radiocarbon Measurements. Journal of Archaeological Science 26: 687–695.

207. Nehlich O, Richards M (2009) Establishing collagen quality criteria for sulphur isotope analysis of archaeological bone collagen. Archaeological and Anthropological Sciences 1: 59–75.

208. Szpak P (2011) Fish bone chemistry and ultrastructure: implications for taphonomy and stable isotope analysis. Journal of Archaeological Science 38: 3358–3372.

209. O'Connell TC, Hedges REM, Healey MA, Simpson AHRW (2001) Isotopic Comparison of Hair, Nail and Bone: Modern Analyses. Journal of Archaeological Science 28: 1247–1255.

210. Towle MA (1961) The Ethnobotany of Pre-Columbian Peru. Chicago: Aldine.

211. Choi W-J, Chang SX, Ro H-M (2005) Seasonal Changes of Shoot Nitrogen Concentrations and ^{15}N/^{14}N Ratios in Common Reed in a Constructed Wetland. Communications in Soil Science and Plant Analysis 36: 2719–2731.

212. Kolb KJ, Evans RD (2002) Implications of leaf nitrogen recycling on the nitrogen isotope composition of deciduous plant tissues. New Phytologist 156: 57–64.

213. Näsholm T (1994) Removal of nitrogen during needle senescence in Scots pine (Pinus sylvestris L.). Oecologia 99: 290–296.

214. Shimada M, Shimada I (1985) Prehistoric llama breeding and herding on the north coast of Peru. American Antiquity 50: 3–26.

215. DeNiro MJ (1988) Marine food sources for prehistoric coastal Peruvian camelids: isotopic evidence and implications. British Archaeological Reports, International Series 427. In: Wing ES, Wheeler JC, editors. Economic Prehistory of the Central Andes. Oxford: Archaeopress. pp. 119–128.

216. Masuda S (1985) Algae Collectors and *Lomas*. In: Masuda S, Shimada I, Morris C, editors. Andean Ecology and Civilization: An Interdisciplinary Perspective on Andean Ecological Complementarity. Tokyo: University of Tokyo Press. pp. 233–250.

217. Wainright SC, Haney JC, Kerr C, Golovkin AN, Flint MV (1998) Utilization of nitrogen derived from seabird guano by terrestrial and marine plants at St. Paul, Pribilof Islands, Bering Sea, Alaska. Marine Biology 131: 63–71.

218. Peterson TC, Vose RS (1997) An Overview of the Global Historical Climatology Network Temperature Database. Bulletin of the American Meteorological Society 78: 2837–2849.

219. Bode A, Alvarez-Ossorio MT, Varela M (2006) Phytoplankton and macrophyte contributions to littoral food webs in the Galician upwelling estimated from stable isotopes. Marine Ecology Progress Series 318: 89–102.

220. Corbisier TN, Petti MV, Skowronski RSP, Brito TS (2004) Trophic relationships in the nearshore zone of Martel Inlet (King George Island, Antarctica): $\delta^{13}C$ stable-isotope analysis. Polar Biology 27: 75–82.

221. Filgueira R, Castro BG (2011) Study of the trophic web of San Simón Bay (Ría de Vigo) by using stable isotopes. Continental Shelf Research 31: 476–487.

222. Fredriksen S (2003) Food web studies in a Norwegian kelp forest based on stable isotope ($\delta^{13}C$ and $\delta^{15}N$) analysis. Marine Ecology Progress Series 260: 71–81.

223. Gillies C, Stark J, Smith S (2012) Small-scale spatial variation of $\delta^{13}C$ and $\delta^{15}N$ isotopes in Antarctic carbon sources and consumers. Polar Biology 35: 813–827.

224. Golléty C, Riera P, Davoult D (2010) Complexity of the food web structure of the *Ascophyllum nodosum* zone evidenced by a $\delta^{13}C$ and $\delta^{15}N$ study. Journal of Sea Research 64: 304–312.

225. Grall J, Le Loc'h F, Guyonnet B, Riera P (2006) Community structure and food web based on stable isotopes ($\delta^{15}N$ and $\delta^{13}C$) analysis of a North Eastern Atlantic maerl bed. Journal of Experimental Marine Biology and Ecology 338: 1–15.

226. Kang C-K, Choy E, Son Y, Lee J-Y, Kim J, et al. (2008) Food web structure of a restored macroalgal bed in the eastern Korean peninsula determined by C and N stable isotope analyses. Marine Biology 153: 1181–1198.

227. Mayr CC, Försterra G, Häussermann V, Wunderlich A, Grau J, et al. (2011) Stable isotope variability in a Chilean fjord food web: implications for N- and C-cycles. Marine Ecology Progress Series 428: 89–104.

228. Nadon MO, Himmelman JH (2010) The structure of subtidal food webs in the northern Gulf of St. Lawrence, Canada, as revealed by the analysis of stable isotopes. Aquatic Living Resources 23: 167–176.

229. Olsen YS, Fox SE, Teichberg M, Otter M, Valiela I (2011) $\delta^{15}N$ and $\delta^{13}C$ reveal differences in carbon flow through estuarine benthic food webs in response to the relative availability of macroalgae and eelgrass. Marine Ecology Progress Series 421: 83–96.

230. Riera P, Escaravage C, Leroux C (2009) Trophic ecology of the rocky shore community associated with the *Ascophyllum nodosum* zone (Roscoff, France): A $\delta^{13}C$ vs $\delta^{15}N$ investigation. Estuarine, Coastal and Shelf Science 81: 143–148.

231. Schaal G, Riera P, Leroux C (2009) Trophic significance of the kelp *Laminaria digitata* (Lamour.) for the associated food web: a between-sites comparison. Estuarine, Coastal and Shelf Science 85: 565–572.

232. Schaal G, Riera P, Leroux C (2010) Trophic ecology in a Northern Brittany (Batz Island, France) kelp (*Laminaria digitata*) forest, as investigated through stable isotopes and chemical assays. Journal of Sea Research 63: 24–35.

233. Schaal G, Riera P, Leroux C (2012) Food web structure within kelp holdfasts (*Laminaria*): a stable isotope study. Marine Ecology 33: 370–376.

234. Vizzini S, Mazzola A (2003) Seasonal variations in the stable carbon and nitrogen isotope ratios ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) of primary producers and consumers in a western Mediterranean coastal lagoon. Marine Biology 142: 1009–1018.

235. Wang WL, Yeh HW (2003) $\delta^{13}C$ values of marine macroalgae from Taiwan. Botanical Bulletin of Academia Sinica 44: 107–112.