Influence of seabird guano and camelid dung fertilization on the nitrogen isotopic composition of field-grown maize (Zea mays)

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Abstract

Organic fertilizers have the capacity to alter the nitrogen isotopic composition of plants. Camelid dung and seabird guano are two potentially important fertilizers in the agricultural systems of western South America, particularly Peru and Chile. This paper presents isotopic data (δ13C and δ15N) from field grown plants (maize, Zea mays) fertilized with the following four treatments: CO (control, no fertilizer applied), AS (ammonium sulfate, a chemical fertilizer), DU (camelid dung), and SG (seabird guano). Plants were grown in experimental plots in the Virú Valley, northern Peru. Plants fertilized with the chemical fertilizer presented very similar isotopic compositions compared to the control. Conversely, the camelid dung fertilized plants were characterized by higher δ15N values compared to the control plants (by 1.8 to 4.2‰ depending on the plant part). The seabird guano fertilized plants were greatly enriched in 15N in comparison to the control plants (by 11.3 to 20.0‰). The results of this study have important implications for the reconstruction of human diet using isotopic data derived from bone collagen and related tissues, particularly in the prehistoric Andes, but also in Europe and North America during the 19th century, when Peruvian seabird guano was used extensively. Specifically, the interpretation of the relative contributions of plant and animal protein to the diet on the basis of bulk isotopic compositions of bone collagen (or similar tissues) may be confounded by camelid dung fertilization if the carbon isotopic compositions of the two sources are similar. Likewise, the interpretation of the relative contributions of maize and marine protein may be confounded by seabird guano fertilization.

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1. Introduction

The reconstruction of diet in archaeological contexts has increasingly relied on stable isotope analysis of human remains. Crucial to this process is a thorough understanding of the range and variation in the isotopic compositions of the foods that may have been consumed (Schwarcz, 1991). The processes influencing the stable nitrogen isotopic compositions (δ15N) in biological systems are complex and are only beginning to be understood. Traditionally, in archaeology and related disciplines, variation in δ15N values of bone collagen and other tissues has been interpreted within the contexts of marine vs. terrestrial resource consumption (e.g. Ambrose et al., 1997; Coltrain, 2009; Richards and Hedges, 1999), animal vs. plant protein consumption (e.g. Katzenberg et al., 1995; Richards et al., 2000), the relative timing of nursing and weaning (e.g. Prowse et al., 2008; Schurr, 1997), and climatic conditions (e.g. Richards and Hedges, 2003; Schwarcz et al., 1999; Szpak et al., 2010). A growing body of literature has emerged in recent years demonstrating that organic fertilizers, specifically those derived from animal waste, can cause large enrichments in 15N of plant tissues (e.g. Bogaard et al., 2007; Choi et al., 2006, 2003; del Amor et al., 2008; Flores et al., 2007; Fraser et al., 2011; Lim et al., 2007; Nakano and Uehara, 2007; Nakano et al., 2003; Rapisarda et al., 2005, 2010; Senbayram et al., 2008; Szpak et al., 2012b; Yun and Ro, 2009; Yun et al., 2006, 2011). Following this, the possibility of manuring as a significant influence on plant and consumer δ15N values in prehistoric agropastoral systems has been given some consideration by archaeologists in recent years. Here, we have sought to determine the influence of short-term fertilization of maize with seabird guano, or camelid dung, on maize nitrogen isotopic composition.
Andean fertilizers

2.1. Camelid dung

South American camelids (llama and alpaca) were the only species of large mammal to have been domesticated in the Americas. They were extremely important in every sphere of prehispanic Andean life, serving as an important source of meat, wool, transportation, and dung (reviewed by Bonavia, 2008; Mengoni Goñalons, 2008). Camelid dung is a relatively poorly known fertilizer because it has never been commercially significant, and has been utilized primarily by subsistence farmers in the Andes.

Camelids are considered to be pseudoruminants because they have a three (rather than four) compartmented stomach, and are distinct from pecoran or ‘true’ ruminants in a number of other ways (see Fowler, 2008). Most significantly, camelids lose proportionately less nitrogen in their urine than other ruminants (Duply et al., 1997; Hinderer and Engelhardt, 1975; Warmington et al., 1989). The total nitrogen content of camelid dung has been reported to be between 1.4 and 1.9% (Alvarez and Lidén, 2008a,b, 2009; Alvarez et al., 2006; Davis et al., 2002), which is similar to other mammalian herbivores (Edwards, 1991; Williams and Haynes, 1995).

Both wild and domestic South American camelids are noted for their habit of communal urination and defecation (Franklin, 1982), which facilitates the collection of manure at a centralized location. This behavior is thought to serve some function related to orienting herd members to their home territory, and also facilitates the development of preferential graze resulting from the localized addition of nutrients to the soil (Franklin, 1982). In some areas, it appears that camelid herds are allowed to graze seasonally in agricultural fields after the harvest, depositing their dung on the fields as they clear the stubble (Mitchell, 1991). As Bruno (2008) points out, however, the application of dung to fields may be dependent on purposeful collection because camelids habitually defecate in one particular location.

Based on studies of modern and historic human groups, camelid dung has been used in tuber cultivation in the high altitude altiplano, which is characterized by soils with low N content and low organic matter (Camino and Johns, 1988; Flannery et al., 1989; Garcilaso de la Vega, 1966; Yamamoto, 1985). Camelid dung may have been used more extensively in the Andes prior to the introduction of European domesticates. The prevalence of camelids in the Andes has markedly declined in the last five hundred years (Mengoni Goñalons, 2008), and the introduction of European livestock (particularly cattle and sheep) has introduced new sources of manure that would not have been available prior to the sixteenth century. For example, in the southern Peruvian altiplano, Winterhalder et al. (1974) found that despite being locally abundant, camelid dung is not often used as a fertilizer, and is instead used primarily for fuel; sheep or cattle manure are the preferred animal fertilizers. There are many more ethnographic and ethnohistoric accounts of the use of camelid dung for a fuel in ceramic production and cooking (Arnold, 1993, 1988; Bruno, 2008; Chávez, 1984–1985; Deere, 1990; Franke, 1992; Hastorf and Wright, 1998; Johannessen and Hastorf, 1990; Silar, 2000a,b), but comparatively fewer accounts of the use of camelid dung as a fertilizer.

2.2. Seabird guano

For the purpose of this paper, ‘guano’ refers specifically to seabird excrement, and not the excrement of bats or pinnipeds, which is commonly also referred to as guano. In very general terms, seabird guano has been the subject of a fairly large body of scientific research because soils that are significantly impacted by seabird guano (ornithogenic soils) are rather unusual in that the concentration of some nutrients (NO$_3^-$ and NH$_4^+$ in particular) may be extremely high and the biota that live in and around these soils are subsidized largely or wholly by seabird excrement (Ellis, 2005; Sánchez-Piñero and Polis, 2008). Seabird guano typically contains 8–21% nitrogen by mass, which is in turn composed primarily of uric acid (~80%), protein (~10%), ammonia (~7%), and nitrate (~0.5%) (Gaskell and Smith, 2007; Gillham, 1960; Hartz and Johnstone, 2006; Lindeboom, 1984; McNabb et al., 1980; Mizutani and Wada, 1985; Mizutani et al., 1991; Staunton Smith and Johnson, 1995; Szpak et al., 2012b).

Mined primarily from small, nearshore islands off the arid western coast of South America in the Peru-Humboldt upwelling region (Fig. 1), seabird guano was the world’s most economically significant fertilizer prior to the twentieth century. Although seabird colonies are present in many other regions in South America, and in some cases provide guano that may be utilized as a fertilizer (e.g. Frere et al., 2005), the Peruvian deposits are by far the best known and were historically considered to be of the highest quality (Cushman, 2003; Hollett, 2008). The Peruvian guano islands are composed of rocky andesite cliffs, which are devoid of vascular plants (Duffy, 1994). The primary guano-producing seabirds of the Peruvian coast (hereafter simply ‘guano birds’) are the Guanay cormorant (Phalacrocorax bougainvillii) and Peruvian booby (Sula variegata), and to a lesser extent the Peruvian brown pelican (Pelecanus occidentalis thagus) (Duffy, 1983a,b, 1994). The guano birds are supported primarily by the Peruvian anchovy and anchoveta (Engraulis ringens), as well as a number of other small mesopelagic fish (Duffy, 1983c; Ludynia et al., 2010; Pauly and Tsukayama, 1987). The Peru-Humboldt upwelling region is the largest producer of fish biomass in the world (Bakun and Broad, 2003; Chavez et al., 2008), which supports the very large populations of nesting seabirds. Since the catastrophic 1965 El Niño and the 1972 collapse of the anchoveta fishery (Muck and Pauly, 1987), which resulted in massive guano bird mortality, Peruvian guano bird populations have remained at less than five million (Tovar et al., 1987). In the 1950s and early 1960s, populations were at their highest recorded levels, fluctuating between ten and thirty million (Duffy, 1983a; Tovar et al., 1987), largely as a consequence of variations in upwelling conditions and primary productivity (Jahnecke et al., 2004). No data or estimates are available for seabird populations in Peru prior to the twentieth century, but it is widely held that the extraction of guano during the nineteenth century (often performed with dynamite) was extremely disruptive to the birds (Tovar et al., 1987). It is likely, therefore, that guano bird populations were even higher prior to the nineteenth century than they were in the twentieth century.

Because the coast of Peru only receives precipitation in exceptional circumstances (during El Niño events), guano accumulates at seabird nesting sites in sedimentary layers. The once thick deposits of seabird guano were removed during the guano boom of the nineteenth century, and today most islands have only several years worth of guano (Duffy, 1994). The guano boom peaked during the middle of the nineteenth century, with millions of tons being exported to North America and Europe (principally the United States and Britain) between the 1830s and the beginning of War in the Pacific (1879) (Murphy, 1981).

The extent to which guano was utilized as a fertilizer prior to the nineteenth century is difficult to assess, but is discussed in various ethnohistorical documents (e.g. Arriaga, 1968; Cieza de León, 1964; Diez de San Miguel, 1964; Garcilaso de la Vega, 1966; Ruiz, 1998; Tschudi, 1854), some of which are described at length by Julien (1985). According to Pedro de Cieza de León (1964: pp. 265–266), writing in the middle of the sixteenth century, the guano was obtained using balsa rafts and applied to the fields, and was crucial to the harvest of a fruitful crop of maize in northern Chile. In the early part of the seventeenth century, Garcilaso de la Vega (1966: p. 246)
wrote, “on the seacoast, from below Arequipa to Tarapacá, a distance of over 200 leagues along the coast, they use no other manure but the dung of sea birds...In the times of the Inca kings these birds were so carefully watched that no one was allowed to land on the [guano] islands during the breeding season under pain of death.” With respect to islands on the south coast of Peru, Netherly (1977) suggests that during the Inca period, highland groups were heavily involved in exploiting guano as fertilizer.

Less is known about the use of seabird guano on the central and north coast of Peru historically, although a number of artifacts and offerings (Fig. 2) were recovered from stratified guano deposits on the northern islands (Macabi, Guañape) during the guano rush of the nineteenth century (Ashmead, 1903; Kubler, 1948). Most of these artifacts display characteristic Moche elements (ca. 100 to 800 AD) and were recovered at significant depths in the guano deposits (for an extensive discussion on Moche material culture, see Bourget and Jones, 2008; Chapdelaine, 2011; Quilter and Castillo, 2010). Therefore, these islands were likely utilized much earlier than the 1500s, although it is unclear to what end: mining guano, fishing camps, hunting guano birds, ritual activity, or a combination of some or all of these. Many authors have speculated on the potential importance of the guano islands (e.g. Benson, 1972, 1995; Covey, 2000; Fonseca and Richardson, 1978; Hocquenghem, 1977; Netherly, 1977; Shimada, 1987, 1994), but the use of guano has been difficult to demonstrate. If the effects of seabird guano fertilization on the isotopic composition of plants can be understood, it may be possible to assess the potential importance of guano as a fertilizer through the isotopic analysis of human or animal remains, and potentially archaeobotanical remains (Aguilera et al., 2008; DeNiro and Hastorf, 1985; Fiorentino et al., 2012; Lightfoot and Stevens, 2012) or sediment profiles (Griffiths et al., 2010; Liu et al., 2006; Yuan et al., 2010).

3. Factors affecting the nitrogen isotopic composition of terrestrial plants

A number of environmental and physiological factors influence the nitrogen isotopic composition of plant tissues (reviewed by

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**Fig. 1.** Map depicting the Peruvian guano islands. The experimental field used in this study is indicated by an arrow.
Craine et al., 2009; Högb erg, 1997; Kohl and Shearer, 1995; Robinson, 2001; Yoneyama, 1995). Plants utilize several forms of inorganic nitrogen, primarily ammonium (NH$_4^+$) and/or nitrate (NO$_3^-$), but also gaseous nitrogen (N$_2$) in some species (e.g. legumes). The extent to which plants rely on these various N sources is significant because the processes that lead to their production in the soil (ammonification, nitrification) and their assimilation into organic N within plants are associated with different fractionation factors (Högberg, 1997; Robinson, 2001). Moreover, because of the differential capacity for NO$_3^-$ to be assimilated in the root or the shoot (NH$_4^+$ is assimilated only in the root), the extent to which plants rely on these sources may also play an important role in the extent of intraplant isotopic variability (Evans et al., 1996). When NO$_3^-$ is immediately assimilated into organic N in the root, any remaining NO$_3^-$ routed to the shoot for assimilation has already been exposed to an assimilatory event that is associated with isotopic fractionation. Therefore, NO$_3^-$ that is assimilated in the shoot is relatively enriched in $^{15}$N compared to NO$_3^-$ assimilated in the root. Shoot $^{15}$N values should be higher than root $^{15}$N values in NO$_3^-$ fed plants.

Aside from the N source, a number of other factors play important roles in determining the nitrogen isotopic composition of plant tissues. Throughout a growing season, or the course of their lives, plants will route and re-route nitrogen contained in their tissues depending on, for example, their growth stage (e.g. vegetative vs. reproductive) or environmental conditions (e.g. onset of winter) (Bausenwein et al., 2001a, 2001b; Evans, 2001; Kolb and Evans, 2002). This is true for maize, with a large portion (60 to 85%) of nitrogen within the plant being reallocated to the grain during ear production (Ta, 1991; Ta and Weiland, 1992). This partitioning and movement of nitrogenous compounds may cause intraplant variation in isotopic composition because the biochemical processes that are involved (protein hydrolysis, protein synthesis) are associated with isotopic fractionations (Bada et al., 1989; Silfer et al., 1992). Therefore, it cannot be assumed that the isotopic composition of any single tissue in a plant necessarily reflects the isotopic composition of the entire plant or its N source.

Animal manures have the capacity to significantly alter the nitrogen isotopic composition of soils and plants (e.g. Bogaard et al., 2007; Choi et al., 2006, 2003, 2002; del Amor et al., 2008; Flores et al., 2007; Fraser et al., 2011; Lim et al., 2007; Nakano and Uehara, 2007; Nakano et al., 2003; Rapisarda et al., 2005, 2010; Senbayram et al., 2008; Szpak et al., 2012b; Yun and Ro, 2009; Yun et al., 2006, 2011). Specifically, plants grown in fields with animal manures tend to have higher tissue $^{15}$N values than plants grown in unfertilized fields, or fields amended with chemical fertilizers such as ammonium sulfate, ammonium nitrate, or urea. The extent to which the fertilizer will impact the tissue $^{15}$N values of the unamended, or manured, fields will depend on a number of factors, including: (1) the diet of the animal, (2) the relative amounts of different N bearing compounds in the manure, (3) whether or not the manure has been composted, (4) the amount of time the manure has been composted, and (5) whether the composting occurred under aerobic or anaerobic conditions.

4. Materials and methods

4.1. Field conditions

We formed a farmer-researcher partnership (Karlen et al., 1995; Katsvairo et al., 2003) with a local farmer (Jorge Rodrigues Paredez), in order to construct a field-scale study on a small farm near Huancaco in the Virú Valley, northern Peru (Fig. 1; 8°27’36”S, 78°48’14”W). The field has been cultivated since 2000, with a variety of crops being grown (asparagus, maize, alfalfa, sugar cane, squash). Prior to 2000, when an irrigation canal was
constructed, the area had no access to water and was not cultivated. No evidence of prehistoric irrigation canals exist and it is therefore unlikely that this particular area was cultivated for at least the last several hundred years. Animals are not encouraged to graze these fields, although it is possible that small numbers of goats may have passed through the area prior to it being opened up for cultivation in 2000. We therefore do not expect an appreciable contribution of animal fertilizers not associated with this experiment to the field. The farmer who cultivates the field has used a chemical fertilizer (ammonium sulfate) and not animal manure. It is thus possible that some N derived from previous chemical fertilizer applications may have been present in the soil during the course of this experiment. The majority of residual N from chemical fertilizer applications, however, tends to be quickly immobilized, with only a small amount (~5%) being present in mineralized form and available for plant uptake (Olson, 1980). Additionally, leaching of chemical fertilizer N as NO$_3^-$ is recognized as a major problem (Costa et al., 2003; Rozas et al., 2004).

Maize used in this experiment is a variety commonly grown on the coast of Peru and is known locally as diente de mula. Seeds were planted on April 28, 2010 and maize was harvested on October 4, 2010. All numeric dates referred to hereafter (e.g. 35 d) represent days elapsed after planting. Four plots were separated based on the type of fertilizer used: AS (ammonium sulfate), CO (control, no fertilizer), DU (camelid dung), and SG (seabird guano) (Fig. 3). Each of the four plots was 36 m$^2$ ($6 \times 6$ m), and was fed by a separate irrigation channel; irrigation was the only source of water throughout the course of the experiment. Plots were separated by raised furrows to minimize the exchange of materials. The experimental area was previously part of a single zone of cultivation, with the four different plots being constructed for the purposes of this experiment. The farmer who owned and raised the diente de mula fields, although it is possible that small numbers of goats may have passed through it in the past, was not encouraged to graze these fields.
characterized by a large equilibrium fractionation ($\delta^{15}N$) for most avian tissues is estimated to be 3 to 4‰ (Caut et al., 2009; Hobson, 1995; Hobson and Clark, 1992). (B) The primary N-bearing molecule in bird excreta is uric acid (C$_5$H$_4$N$_4$O$_3$) (Wainright et al., 1998). Seabird guano (bulk) is ~2.5‰ depleted of $\delta^{15}N$ relative to blood (Wainright et al., 1998). Based on the observations of several investigators (Bird et al., 2008; Mizutani et al., 1985a; Mizutani et al., 1985b) uric acid and bulk guano $\delta^{15}N$ are very similar to one another. (C) Uric acid is rapidly mineralized to ammonium in the soil (Kirchmann, 1991); the fractionation of $\delta^{15}N$ is from Mizutani et al. (1985a). (D) Volatilization of ammonium to gaseous ammonia, which is known to be characterized by a large equilibrium fractionation (~25 to ~60‰), which results in the remaining substrate ($^{15}NH_4^+$ in the diagram) being comparatively enriched in $\delta^{15}N$ by ~40‰ (Mizutani et al., 1985b; Mizutani et al., 1986). (E) The fractionation of $^{15}N$ associated with nitrification (oxidation of ammonium to nitrate) has been estimated at between ~12 and ~35‰ (Robinson, 2001; Shearer and Kohl, 1986). (F) Depending on the extracellular concentration of ammonium, its uptake is associated with a nitrogen isotope fractionation ranging from ~6 to ~30‰ (Hoch et al., 1992; Yoneyama et al., 2001). The nitrogen isotopic composition of the ammonium taken up by the plant will vary over time depending on the speed with which $NH_3$ volatilization and nitrification take place. (G) There is no fractionation associated with the uptake of nitrate (Marriott et al., 1982; Yoneyama et al., 1998; Yoneyama et al., 2001). Ammonium and nitrate may be effluxed from the plant, depending on environmental and physiological conditions (see Miller and Cramer, 2005).

cultivated this field and the surrounding area. Fertilizers were applied after seedling establishment because seabird guano has the potential to inhibit seedling germination and establishment (Szpak et al., 2012b).

Seabird guano was purchased at a local market in Peru and camelid dung was collected from a pasture north of Sausacocha Lake near Huamachuco (Fig. 1). Only alpacas were observed depositing dung on this pasture, but because it is possible that a small amount of llama dung may also have been included, we refer to the dung as ‘camelid’.

Temperature and relative humidity were typical for northern Peru during the study period; no rain events were recorded during the study period.

Leaf samples were taken from five plants selected at random twice a month (on the 15th and last day of each month) beginning 21 days after planting. At the conclusion of the experiment, stalks and grains were sampled from eight plants.

4.2. Isotopic methodology

All plant materials were air-dried on site at room temperature following sampling. Samples were then air-dried at 90°C for at least 72 h in the laboratory. Following this, samples were ground using a Wig-L-Bug (Crescent) and stored at room temperature in sealed glass vials.

Five aliquots of ~25 g each of ammonium sulfate, camelid dung and seabird guano were ground to fine powders using a mortar and pestle. The purpose of the analysis of these aliquots was to attempt to account for any variability in the nitrogen isotopic compositions of the fertilizers. This material was air-dried as described above prior to isotopic analysis. Nitrogen isotopic compositions and elemental nitrogen contents were determined using a Thermo Finnigan Delta V isotope ratio mass spectrometer coupled to a Costech elemental analyzer. For the analysis of $\delta^{15}N$, excess CO$_2$ was removed with a Carbo-Sorb® trap (Elemental Microanalysis). Sample reproducibility was ±0.09‰ for $\delta^{15}N$ (21 replicates), and ±0.08 for %N (20 replicates). A $\delta^{15}N$ value of 6.31 ±0.11‰ was obtained for 75 analyses of an internal keratin standard calibrated against international standards USGS-40 (glutamic acid) and USGS-41 (glutamic acid), which compared well with its average value of 6.36‰.

4.3. Statistical analyses

Differences in isotopic and elemental compositions between treatments and plant parts were assessed with a one-way analysis of variance (ANOVA). Homogeneity of variance was assessed using Levene’s test, and a post hoc Tukey’s honestly significant difference (HSD) statistic was applied if variance was homoscedastic, or Dunnett’s T3 test was applied if variance was not homoscedastic. For all statistical analyses, a significance level of $p < 0.05$ was used. Statistical analyses were performed in SPSS 16.

5. Results and discussion

5.1. Nitrogen isotopic composition of fertilizers

The $\delta^{15}N$ values for the three fertilizers used in this study were significantly different from one another ($F_{2,12} = 7977.5, p < 0.001$): ammonium sulfate ~0.7 ± 0.1‰, camelid dung 13.9 ± 0.6‰, seabird guano 38.1 ± 0.6‰. All data for fertilizers are listed in the Supplementary Material (Table S1). The low $\delta^{15}N$ value of the ammonium sulfate is typical of chemical fertilizers, which tend to have nitrogen isotopic compositions close to 0‰ (Bateman and Kelly, 2007;
This process is much more significant in the vicinity of seabird nesting sites (Lindeboom, 1984; Zhu et al., 2011). As discussed above, seabird guano, and the excreta of all birds, is compositionally very different than mammalian excreta. It is a mixture of urine and feces, which tends to contain proportionately more nitrogen on a dry weight basis, with the bulk of this nitrogen as uric acid (Nicholson et al., 1996; Wright, 1995). This is significant because uric acid is very rapidly and completely mineralized in soils to ammonium (Kirchmann, 1991). In seabird rookeries, much of this ammonium (possibly >75%; Lindeboom, 1984) is lost through volatilization to gaseous ammonia. As discussed above, this process is associated with a large fractionation, which leaves the remaining ammonium highly enriched in $^{15}$N. Therefore, the

<table>
<thead>
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<th>Treatment</th>
<th>Tissue</th>
<th>Sampling date (d)</th>
<th>$\delta^{15}$N (permil, AIR)</th>
<th>%N</th>
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<tbody>
<tr>
<td>CO</td>
<td>Leaf</td>
<td>18</td>
<td>6.0 ± 1.8</td>
<td>5.6 ± 0.9</td>
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<td>Leaf</td>
<td>31</td>
<td>6.4 ± 2.1</td>
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<tr>
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<td>Leaf</td>
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<tr>
<td></td>
<td>Grain</td>
<td>157</td>
<td>6.3 ± 0.3</td>
<td>1.9 ± 0.1</td>
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<tr>
<td></td>
<td>Stalk</td>
<td>157</td>
<td>9.8 ± 0.6</td>
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<td>31</td>
<td>23.1 ± 0.6</td>
<td>4.6 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>Leaf</td>
<td>46</td>
<td>30.9 ± 6.4</td>
<td>2.9 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>Leaf</td>
<td>61</td>
<td>30.4 ± 0.2</td>
<td>4.0 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>Leaf</td>
<td>76</td>
<td>30.1 ± 3.9</td>
<td>3.6 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>Leaf</td>
<td>91</td>
<td>28.0 ± 5.5</td>
<td>3.2 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Leaf</td>
<td>107</td>
<td>30.4 ± 2.4</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Leaf</td>
<td>122</td>
<td>25.4 ± 3.9</td>
<td>1.9 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>Leaf</td>
<td>138</td>
<td>23.7 ± 2.0</td>
<td>2.0 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>Grain</td>
<td>157</td>
<td>21.2 ± 0.2</td>
<td>1.5 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Stalk</td>
<td>157</td>
<td>21.2 ± 3.0</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>AS</td>
<td>Leaf</td>
<td>18</td>
<td>5.1 ± 0.8</td>
<td>5.4 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Leaf</td>
<td>31</td>
<td>1.4 ± 1.0</td>
<td>4.7 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Leaf</td>
<td>46</td>
<td>4.8 ± 2.3</td>
<td>4.2 ± 0.3</td>
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<tr>
<td></td>
<td>Leaf</td>
<td>61</td>
<td>5.9 ± 1.1</td>
<td>4.1 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>Leaf</td>
<td>76</td>
<td>4.2 ± 3.5</td>
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<td>Leaf</td>
<td>91</td>
<td>4.5 ± 1.4</td>
<td>3.5 ± 0.2</td>
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<tr>
<td></td>
<td>Leaf</td>
<td>107</td>
<td>3.4 ± 2.0</td>
<td>2.9 ± 0.4</td>
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<tr>
<td></td>
<td>Leaf</td>
<td>122</td>
<td>4.9 ± 1.0</td>
<td>2.7 ± 0.2</td>
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<tr>
<td></td>
<td>Leaf</td>
<td>138</td>
<td>5.0 ± 0.8</td>
<td>2.7 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Grain</td>
<td>157</td>
<td>5.8 ± 0.2</td>
<td>1.6 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>Stalk</td>
<td>157</td>
<td>13.7 ± 3.2</td>
<td>1.2 ± 0.4</td>
</tr>
</tbody>
</table>

### Table 2

Results of ANOVA for differences in nitrogen isotopic and elemental compositions between treatments.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Treatment</th>
<th>SG</th>
<th>AS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf (138 d)</td>
<td>CO</td>
<td>0.004</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>DU</td>
<td>&lt;0.001</td>
<td>0.055</td>
</tr>
<tr>
<td></td>
<td>SG</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>AS</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Grain (157 d)</td>
<td>CO</td>
<td>&lt;0.001</td>
<td>0.027</td>
</tr>
<tr>
<td></td>
<td>DU</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>SG</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>AS</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Stalk (157 d)</td>
<td>CO</td>
<td>0.631</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>DU</td>
<td>0.004</td>
<td>0.944</td>
</tr>
<tr>
<td></td>
<td>SG</td>
<td>&lt;0.001</td>
<td>0.007</td>
</tr>
</tbody>
</table>

Values in boldface are statistically significant (p < 0.05).
guano collected from the islands was likely subjected to ammonia volatilization and subsequent enrichment in $^{15}\text{N}$ prior to collection. That said, ammonia volatilization occurs much more rapidly in moist soil (Ernst and Massey, 1960; Ferguson and Kissel, 1985), and it is thus unlikely to have been as significant as in other wetter environments because of the hyper-arid conditions of the Peruvian guano islands.

The nitrogen isotopic compositions of animal fertilizers are highly variable and are influenced by a number of factors other than the diet or trophic level of the animal. As discussed above, the isotopic composition of manure depends on the relative proportions of N bearing compounds in the manure (organic N, urea, uric acid, nitrate, ammonia), the speed at which they are mineralized (for organic N), and the fractionations associated with their decomposition in the soil.

The detection of fertilization practices using isotopic analysis is complicated by the fact that the isotopic composition of manure can change over time. These changes may occur after the manure is applied to the soil, and/or prior to the application of manure to the soil. Several authors have noted that composting significantly impacts the $^{15}\text{N}$ values of animal manures (Hristov et al., 2006, 2009; Lee et al., 2011). The type of bedding material used may also impact composted manure $^{15}\text{N}$ values (Kim et al., 2008). Further investigation and consideration of the elemental and isotopic compositions of a wider range of fertilizers that may have been important in antiquity is crucial. Equally as important, however, is a thorough understanding of the availability of various N bearing species from manures (cf. Choi et al., 2006).

5.2. Nitrogen isotopic composition of fertilized plant tissues

Mean nitrogen isotopic compositions and elemental nitrogen contents for plant tissues sampled at harvest, as well as leaves sampled throughout the course of the experiment are presented in Table 1. Individual results for all plant tissues are presented in the Supplementary material (Table S1). There were significant differences in $^{15}\text{N}$ values between the SG and CO treatments for all tissues sampled, and between DU and CO for grain and leaves, but not for stalk (Table 2, Fig. 6). The AS plants had significantly lower grain $^{15}\text{N}$ values than the CO plants, but stalk and leaf $^{15}\text{N}$ value did not differ significantly. The magnitude of the difference in $^{15}\text{N}$ between the SG and CO plants ($\Delta^{15}\text{N}_{\text{SG} - \text{CO}}$ for grain, 11.3‰ for stem, 20.0‰ for leaves at 138 d) was much greater than between DU and CO ($\Delta^{15}\text{N}_{\text{DU} - \text{CO}}$ for grain, 2.3‰ for stem, 4.2‰ for leaves at 138 d) (Fig. 6).

While the nitrogen isotopic composition of AS grain was significantly lower than the CO grain, the magnitude of this difference was only 0.5‰. A lack of differentiation in $^{15}\text{N}$ values between control plants and plants fertilized with ammonium sulfate has been observed in other studies examining the effect of chemical fertilizers on plant nitrogen isotopic composition. Kriszan et al. (2009) found no significant differences in $^{15}\text{N}$ between plants grown with no fertilizer or with ammonium sulfate in a temperate grassland. Similarly, Yun et al. (2011) found no significant difference in $^{15}\text{N}$ between ammonium sulfate fertilized and control plants for rice (Oryza sativa L.) roots and grains, but a significant difference for shoots ($\sim 3$‰).

The camelid dung fertilized maize was characterized by higher $^{15}\text{N}$ values relative to the control maize for all tissues analyzed. The magnitude of the difference in $^{15}\text{N}$ between the CO and the DU plant tissues ($1.8$–$4.2$‰) is comparable to other studies that have utilized mammalian manures (Bateman et al., 2005; Fraser et al., 2011; Lim et al., 2007; Nakano and Uehara, 2007; Yun et al., 2011). The pattern observed in this study demonstrates significant uptake of camelid dung-derived N by maize after a single season of fertilization at a relatively low application rate (75 kg N ha$^{-1}$).

It is possible that long-term application of this manure to agricultural fields and/or application at a higher rate would result in a greater increase in plant $^{15}\text{N}$. Fraser et al. (2011) observed that the distinction in plant $^{15}\text{N}$ values between fertilized (cattle manure) and unfertilized plots was much greater in long-term (manure applied for several years or decades) than in short-term (single application of manure) studies. Specifically, they suggested a ‘pronounced manuring effect’ becomes apparent after a decade of plot establishment. Other studies have demonstrated greater increases in plant $^{15}\text{N}$ values with higher application rates of organic fertilizers (Yun and Ro, 2009) because the availability of N derived from organic fertilizers tends to increase with the rate of application (Burger and Venterea, 2008; Habteselasie et al., 2006). This is true both in the short term because of the rapid release and mineralization of labile N, and in the long term because of the slow release of manure N, which was initially immobilized, over a period of many years (Burger and Venterea, 2008; Sørensen and Amato, 2002). Additionally, the influence of urinary N that has mixed with fecal N at camelid defecation sites may play an important role in the immediate ‘manuring effect’ that was observed in the nitrogen isotopic composition of maize in this study. This N, which is rapidly mineralized, may provide an initial pulse of inorganic N for plants shortly after fertilizer application.

As was the case with the DU plants, there was a significant difference in plant tissue $^{15}\text{N}$ values between the SG and CO maize, although the magnitude of this difference was much greater. This is partially explained by the very high $^{15}\text{N}$ values of

![Fig. 6. Nitrogen isotopic compositions of maize tissues. Horizontal bars represent means and vertical lines represent standard deviations. Numeric values above AS, DU, and SG data represent differences in $^{15}\text{N}$ relative to CO plants ($\Delta^{15}\text{N}_{\text{Treatment} - \text{Control}}$).](image-url)
the seabird guano (38.1 \pm 0.6\%o), but equally important is the availability of nitrogen from the seabird guano. The rate of application of the two fertilizers (camelid dung and seabird guano) was similar, but because the seabird guano contained approximately three times as much nitrogen as the camelid dung, the rate of application, in terms of total N, was much higher in the seabird guano plot. Moreover, the guano N was more readily available for plant uptake than the camelid dung N. The primary N-bearing compound in bird excrement (uric acid) is rapidly and completely converted to NH$_4^+$, and in turn to NO$_3^-$, such that a very large portion of the guano N is mineralized and available for plant uptake within a few days or weeks of application (Hadas and Rosenberg, 1992; Kirchmann, 1991; Sims and Wolf, 1994). For these reasons, a much greater amount of guano-derived, compared to dung-derived, nitrogen would be available to plants, particularly in the short term.

Fig. 7. Temporal patterns in foliar nitrogen isotopic and elemental composition. (A) Maize leaf $\delta^{15}$N values; each point represents the mean of three randomly sampled plants, with the exception of the last point, which represents six randomly sampled plants; vertical bars represent standard deviations. (B) Leaf N content. (C) Differences between fertilized and control leaf $\delta^{15}$N values for DU (primary y-axis) and SG (secondary y-axis) plants. Broken vertical lines represent fertilizer applications.
5.3. Temporal patterns in foliar $\delta^{15}$N values

The nitrogen isotopic compositions and elemental N concentrations for leaves sampled at two-week intervals are presented in Fig. 7A ($\delta^{15}$N) and 7B ($\%N$). Additionally, the differences between mean nitrogen isotopic compositions relative to the control for camelid dung ($\Delta^{15}$N$_{DU - CO}$) and seabird guano ($\Delta^{15}$N$_{SG - CO}$) are presented in Fig. 7C. There was no clear differentiation in nitrogen isotopic composition between the CO and AS plants, while the DU control plants (Szpak et al., 2012b).

The higher $\delta^{15}$N values for the DU plants in comparison with the CO plants from the first sampling suggest there was mineralized nitrogen (NH$_4^+$ or NO$_3^-$) almost immediately available from the dung for early plant growth as the first sampling took place on three days after the first fertilizer application. This fits with observations concerning cattle dung that demonstrated a rapid release of mineralized N that lasted a few days or weeks (Anttil et al., 2001; Dickinson and Craig, 1990; Lovell and Jarvis, 1996). The fairly consistent foliar $\delta^{15}$N values for DU plants further demonstrates the slow release of initially immobilized N derived from the dung over time, as discussed above.

The SG plants were characterized by the highest $\delta^{15}$N values at every sampling point (Fig. 7A). As was the case with the camelid dung, the seabird guano fertilized plants were characterized by higher $\delta^{15}$N values than the control plants at the first sampling. Initially, there was an increase in $\delta^{15}$N values from ~12$_{\text{swm}}$ to ~30$_{\text{swm}}$ followed by a gradual decrease in $\delta^{15}$N to ~23$_{\text{swm}}$ at the end of the experiment (Fig. 7A). The immediate influence of guano on maize nitrogen isotopic composition reflects the rapid mineralization of uric acid N to NH$_4^+$ in soil. The increase in $\delta^{15}$N values over the following weeks to ~30$_{\text{swm}}$ was likely the result of very high concentrations of NH$_4^+$ in the soil derived from guano, which was subject to further volatilization to NH$_3$, leading to significant $^{15}$N enrichment in the residual NH$_4^+$ pool over time. The decrease in foliar $\delta^{15}$N after 45 d was likely the product of increased uptake of NO$_3^-$ rather than NH$_4^+$, as nitrification proceeded. Nitrification discriminates strongly against $^{15}$N (~15 to ~35$_{\text{swm}}$), resulting in NO$_3^-$ having lower $\delta^{15}$N values than NH$_4^+$ (Feigin et al., 1974; Mariotti et al., 1981; Robinson, 2001; Shearer and Kohl, 1986). The very gradual decline in foliar $\delta^{15}$N values in the SG plants over time is likely the result of a fairly slow rate of nitrification. At high concentrations of soil NH$_4^+$, which are typical in ornithogenic soils, nitrification is inhibited because of the presence of excess soil salts and reduced soil pH (Malhi and McGill, 1982; Shaviv, 1988; Stojanovic and Alexander, 1958). Additionally, processes such as immobilization and leaching of mineral nitrogen derived from bird excrement would serve to decrease the quantity of guano-derived nitrogen available to plants over time (Thomsen, 2004).

Overall, the temporal patterns of foliar $\delta^{15}$N in fertilized plants are difficult to interpret, particularly for short-term experiments. Foliar tissue develops discontinuously through the growth cycle of annuals (such as maize) depending on the stage of growth (vegetative vs. reproductive), environmental conditions, and the nutrient status of the plants. Nutrients, including nitrogen, in tissues such as the stalk and leaves are later remobilized to the reproductive tissues as the plant grows (Beauchamp et al., 1976; Donnison et al., 2007; Masclaux-Daubresse et al., 2008; Ta, 1991; Ta and Weiland, 1992). These factors may, therefore, cause considerable variation in the isotopic composition of plant leaves. Because of this, the isotopic composition of a single leaf sampled at any given time will be influenced by a number of factors other than the availability and isotopic composition of the N source(s).

5.4. Intraplant $\delta^{15}$N variability

The biochemical processes associated with the catabolism and synthesis (e.g. deamination, transamination) of N-bearing species within a plant may be associated with significant fractionations (Macko et al., 1986, 1987; Tcherkez, 2011; Werner and Schmidt, 2002) that should concentrate nitrogen sources (e.g. senescent leaves) in $^{15}$N, and produce nitrogen sinks (e.g. grains) that are comparatively depleted of $^{15}$N (e.g. Choi et al., 2005; Choi et al., 2002; Gebauer et al., 1994; Nåsholm, 1994), although some studies have not observed this effect (e.g. Fraser et al., 2011; Garten, 1993; Kolb and Evans, 2002).

Intraplant differences in $\delta^{15}$N were significant for CO and AS treatments, but not for SG and DU treatments (Table 3). For CO, AS, and DU treatments, the stalk had the highest $\delta^{15}$N values, while the leaves had the lowest $\delta^{15}$N values. No clear pattern of intraplant variability was evident in the SG treatment. The generally high stalk $\delta^{15}$N values relative to the grain (for all treatments) suggests that the stalk serves as an important source for absorbed N that is later reallocated to the grain during ear production, enriching the source (stalk) in $^{15}$N relative to the sink (grain) as described above. This pattern of $^{15}$N enrichment in stalks relative to grains was suggested previously by Szpak et al. (2012b) based on isotopic data from growth chamber grown maize fertilized with seabird guano. More generally, there is a large body of experimental evidence demonstrating the role of the stalk as an important, or dominant, source of absorbed N that is later allocated to the grain during ear development (Rizzi et al., 1991; Subedi and Ma, 2005; Ta, 1991; Ta and Weiland, 1992; Ta et al., 1993).

One of the more important implications of the intraplant variability observed in this study relates to the consumption of different plant parts by humans and animals, respectively. The practice of allowing animals to graze on byproducts in agricultural fields is widely known from modern observations throughout the world (e.g. Goland, 1993; Ibrahim et al., 1988; Miehe, 1986; Salzman, 1971; Wiegars et al., 1999), including for camelids in the Andes (McCorl, 1987; Mitchell, 1991; Nielsen, 2001). In some cases, this act of animal grazing is seen as particularly beneficial because the animal excrement that is left on the fields acts as a fertilizer for the next growing season (Salzman, 1971).

The significant point here is that if animals subsisted to a large extent on agricultural byproducts, it is very possible that this could lead to animals having tissues relatively enriched in $^{15}$N compared to

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tissue</th>
<th>Grain (157 d)</th>
<th>Stalk (157 d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO</td>
<td>Leaf (138 d)</td>
<td>0.002</td>
<td>-0.001</td>
</tr>
<tr>
<td></td>
<td>Grain (157 d)</td>
<td>-</td>
<td>-0.001</td>
</tr>
<tr>
<td>DU</td>
<td>Leaf (138 d)</td>
<td>0.966</td>
<td>0.126</td>
</tr>
<tr>
<td></td>
<td>Grain (157 d)</td>
<td>-</td>
<td>0.099</td>
</tr>
<tr>
<td>SG</td>
<td>Leaf (138 d)</td>
<td>0.070</td>
<td>0.281</td>
</tr>
<tr>
<td></td>
<td>Grain (157 d)</td>
<td>-</td>
<td>0.999</td>
</tr>
<tr>
<td>AS</td>
<td>Leaf (138 d)</td>
<td>0.155</td>
<td>-0.001</td>
</tr>
<tr>
<td></td>
<td>Grain (157 d)</td>
<td>-</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Values in boldface are statistically significant ($p \leq 0.05$).
humans that consumed grain from the same plants. The consumption of these animals by humans adds another complication to the interpretation of the relative contributions of plant and animal protein. The relationship between the isotopic compositions of grains/fruits and leaves/stems requires further exploration before any generalizations can be made in this respect.

5.5. Implications for dietary reconstruction using stable isotopes

On the basis of studies conducted with manures derived from other terrestrial herbivores, differentiating human diets composed primarily of plant or animal protein using stable isotope analysis of bone collagen or similar tissues would be difficult if crops were amended with manure. As discussed by Bogaard et al. (2007), the differentiation of largely plant vs. animal based diets is dependent on the observation that there is a consistent enrichment in $^{15}$N at each trophic level (Caut et al., 2009; Minagawa and Wada, 1984). There is considerable variability in $^{15}$N trophic enrichment, but based on a survey of published literature, Szpak et al. (2012a) found it to be $-3.7 \pm 1.6\%$ for mammalian bone collagen. Therefore, the difference in $^{15}$N values between the CO and DU maize grain ($1.8\%$) is roughly equivalent to half a trophic level, with greater differences for the stem ($2.3\%$) and leaves ($4.2\%$). As discussed previously, it is possible that higher application rates and long-term fertilization could have an even more pronounced effect on plant $^{15}$N values. This implies that in the Andes, the fertilization of crops with camelid dung has the potential to complicate interpretations of the consumption of plant vs. animal protein using bulk isotopic analysis of tissues such as bone collagen. This is a particularly important consideration as the possibility that fertilization with camelid dung was an integral part of the successful wide-scale adoption of maize agriculture in southern Peru (Cuzco area) has recently been suggested (Chepstow-Lusty, 2011). The use of camelid dung could have been much more widespread, however, including lower altitude zones and the coast, where there is evidence for the breeding of camelids (Rostworowski, 1981; Shimada and Shimada, 1985; Wilson, 1988).

A limited number of archaeological camelids from coastal and low altitude sites that have been subjected to isotopic analysis are characterized by relatively high $^{15}$N values ($>10\%e$), which are thought to be indicative of the consumption of marine resources, llamas, or fertilized plants (DeNiro, 1988; Thornton et al., 2011). The consumption of self-fertilized wild vegetation or crops by camelids is certainly possible and may partially explain these relatively high $^{15}$N values. Another very plausible explanation for these unusually high $^{15}$N values is related to the arid nature of much of the Peruvian coastal region. It is established that such conditions can cause markedly high plant nitrogen isotopic compositions (Hartman, 2011; Heaton, 1987; Murphy and Bowman, 2006, 2009; Sealy et al., 1987), which are also reflected in local fauna (Ambrose and DeNiro, 1987; Gröcke et al., 1997; Pate and Anson, 2008; Schwarze et al., 1999). Intensive regional sampling

Table 4 Inputs and summarized results for the mixing model presented in Fig. 8.

<table>
<thead>
<tr>
<th>Source</th>
<th>$\delta^{13}$C</th>
<th>$\delta^{15}$N</th>
<th>$\delta^{13}$C</th>
<th>$\delta^{15}$N</th>
<th>% Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD (1st–99th percentile)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Scenario A (no fertilization)

<table>
<thead>
<tr>
<th>Source</th>
<th>$\delta^{13}$C</th>
<th>$\delta^{15}$N</th>
<th>$\delta^{13}$C</th>
<th>$\delta^{15}$N</th>
<th>% Contribution</th>
</tr>
</thead>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

Scenario B (camelid dung fertilization)

<table>
<thead>
<tr>
<th>Source</th>
<th>$\delta^{13}$C</th>
<th>$\delta^{15}$N</th>
<th>$\delta^{13}$C</th>
<th>$\delta^{15}$N</th>
<th>% Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Scenario C (seabird guano fertilization)

<table>
<thead>
<tr>
<th>Source</th>
<th>$\delta^{13}$C</th>
<th>$\delta^{15}$N</th>
<th>$\delta^{13}$C</th>
<th>$\delta^{15}$N</th>
<th>% Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$\delta^{13}$C and $\delta^{15}$N have been adjusted for diet-tissue fractionation ($-3.7$ for $\delta^{13}$C and $+3.6$ for $\delta^{15}$N).
programs of Peruvian plants for isotopic analysis will aid in clarifying these issues.

In comparison to the relatively modest enrichment in $^{15}\text{N}$ in the DU plants, the SG plants were characterized by much higher $^{15}\text{N}$ values. Whereas plants fertilized with camelid dung, or the dung of other mammalian herbivores (e.g., cow, sheep, horse), may be isotopically similar (in terms of $^{15}\text{N}$) to the tissues of such herbivores, the seabird guano fertilized plants exhibited $^{15}\text{N}$ values that are comparable to, and often greater than, high-trophic level marine animals, such as pinnipeds, predatory fish, and piscivorous seabirds (Forero et al., 2004; Mayr et al., 2011; Schoeninger and DeNiro, 1984; Tieszen and Chapman, 1995). Even higher $^{15}\text{N}$ values were observed for guano-fertilized maize grown in containers under controlled conditions (Szpak et al., 2012b).

Maize is a C$_4$ plant, with $^{13}\text{C}$ values in the range of $-15$ to $-10^{\text{ppm}}$ (Jahren et al., 2006; Lasa et al., 2011; Piasentier et al., 2003; Szpak et al., 2012b; Tieszen and Fagre, 1993; White et al., 2001), which are comparable to those of many high trophic level marine animals (Richards and Hedges, 1999; Schoeninger and DeNiro, 1984; Szpak et al., 2009). The use of animal manure fertilizers has been suggested by some investigators to cause plants to have $^{13}\text{C}$-depleted tissues relative to plants grown with chemical fertilizers, although the differences are very small in magnitude ($\leq 1^{\text{ppm}}$; Camin et al., 2011; Georgi et al., 2005; Lim et al., 2007). In many other studies, however, no systematic variation has been observed (Birkhofer et al., 2011; Bol et al., 2005; Camin et al., 2007; Flores et al., 2007; Nakano et al., 2003; Rapisarda et al., 2010; Szpak et al., 2012b).

Those studies that have observed differences in plant $^{13}\text{C}$ values associated with organic fertilization have contrasted organically and inorganically fertilized plants, rather than organically fertilized and control plants. Previously, we found no relationship between maize $^{13}\text{C}$ values and the application of Peruvian seabird guano fertilizer in a growth chamber experiment (Szpak et al., 2012b). There is, therefore, no evidence that suggests plant $^{13}\text{C}$ values would change substantially as a result of fertilization with animal manure.

When maize is fertilized with seabird guano, the carbon and nitrogen isotopic compositions of the plant’s tissues will be very similar to those of high trophic level marine animals. Therefore, in the same way that the differentiation between plant and animal protein is complicated by the possibility of fertilization with livestock manure, the differentiation between terrestrial and marine protein is complicated by the fertilization of maize with seabird guano. This is particularly relevant since many of the areas where guano fertilization is suspected are located on, or in close proximity, to the coast (Bawden, 1996; Benson, 1972, 1995; Hoquequenhem, 1977; Netherly, 1977; Nordt et al., 2004; Shimada, 1987, 1994), and hence, both guano and marine resources would have been accessible.

As a very simple illustrative example, we have applied the Isosource mixing model (inputs: tolerance $= 0.1$, increment $= 1$) presented by Phillips and Gregg (2003) to three different scenarios that differ only in the nitrogen isotopic composition of maize caused by fertilization (Table 4; Fig. 8). In each of the three scenarios, the consumer (represented by a black $\times$ in Fig. 8) has an isotopic composition ($^{13}\text{C} = -10^{\text{ppm}}$, $^{15}\text{N} = 22^{\text{ppm}}$) that would traditionally be interpreted as being indicative of a marine-intensive diet. In Scenario A (no fertilization), the reconstructed diet is dominated by sea lion, followed by sardine; the three terrestrial foods (maize, potato, llama) contribute very little (Table 4). For a consumer with high carbon and nitrogen isotopic compositions (as presented in Fig. 8), the small shift in nitrogen isotopic composition resulting from maize fertilization with camelid dung (Scenario B) has little impact on the relative contributions of the different foods (Fig. 8B). This would not necessarily be true in other contexts where the impact of marine foods is smaller, and the marine animal protein source has a similar carbon isotopic composition to the fertilized maize; this might be expected in the high altitude regions of the Andes, where camelid dung fertilization is suspected to have been important (Chepstow-Lusty, 2011).

In Scenario C (guano fertilization), the relative contributions of the terrestrial foods (llama and potato) remains very low, the mean contributions of the marine foods (sardine and sea lion) remain high, and the mean contribution of maize increases significantly. More important than this, however, is the change that occurs in the shape of the mixing polygon formed by the sources (top panels of Fig. 8). The convergence in $^{15}\text{N}$ values between the maize and the marine sources (particularly the sea lion) causes a narrowing of the polygon, which leads to more diffuse solutions for the sea lion and guano fertilized maize (Fig. 8C). This contrasts to Scenarios A and B (Fig. 8A and B), in which the solutions for maize and sea lion are well constrained. In a practical sense, this simply demonstrates that when maize is fertilized with seabird guano, the quantitative differentiation between maize consumption and marine resource consumption on the basis of bulk tissue isotopic composition becomes more difficult. The application of carbon isotopic analysis of individual amino acids isolated from bone collagen (e.g., Corr et al., 2005) may be necessary to truly differentiate between maize and marine-based diets where the potential use of seabird
guano fertilizer exists, but this avenue of research requires much additional exploration.

While a growing number of studies have examined the isotopic composition of archaeological human (e.g. Finucane et al., 2006; Finucane, 2007; Kellner and Schoeninger, 2008; Knudson et al., 2007; Slovak and Paytan, 2011; Tomczak, 2003; Verano and DeNiro, 1993; White et al., 2008; Williams and Katzenberg, 2012) and animal (e.g. DeNiro, 1988; Finucane et al., 2006; Thornton et al., 2011; Verano and DeNiro, 1993) remains from the Andean region, it is relatively difficult to assess any particular cases that provide direct evidence for the use of seabird guano and/or camelid dung as a fertilizer. Some of the extremely high (>20\%) δ¹⁵N values presented for numerous individuals from several Chiribaya sites in the Osmore drainage of southern Peru (Tomczak, 2003) are certainly suggestive of the potential importance of guano, but as discussed above, the isotopic analysis of bulk tissues such as bone collagen does not provide sufficient resolution to differentiate between the consumption of high trophic level marine resources and guano-fertilized maize. Therefore, both of these alternatives must be considered as potential explanatory factors when interpreting isotopic data from archaeological materials from the Andes.

The uncertainty inherent in the interpretation of isotopic data as discussed above highlights the importance of integrating multiple lines of evidence in the reconstruction of ancient diet, particularly the consideration of relevant zooarchaeological and paleoethnobotanical data. With respect to zooarchaeology in particular, these data can provide basic and very essential information in terms of the presence and abundance of particular taxa in the subsistence economy. In cases where a significant maritime adaptation is evident in the zooarchaeological assemblages (e.g. Marcus et al., 1999; Reitz, 1988, 2001; Sandweiss, 1992; Sandweiss et al., 1989), a more reasonable case can be made for the consumption of marine resources being responsible for particularly high carbon and nitrogen isotopic compositions. With respect to paleoethnobotany, in addition to documenting the presence of particular crops that may have been cultivated and fertilized, the isotopic analysis of macrobotanical remains (Aguilera et al., 2008; Araus et al., 2001, 1999, 1997; DeNiro and Hastorf, 1985; Fiorentino et al., 2012; Heaton et al., 2009; Lightfoot and Stevens, 2012) hold the greatest potential to provide unequivocal evidence for the fertilization of crops. This is especially true for seabird guano because the enrichment in plant tissue ¹⁵N is much larger than what would be expected in unfertilized crops. Conversely, camelid dung fertilization would be more difficult to detect with such analyses due to the much more modest enrichment in plant tissue ¹⁵N. This is particularly true in the dry coastal region of Peru and Chile, where higher plant ¹⁵N values may simply be related to water availability: such effects have been observed throughout the world on many scales (Craine et al., 2009; Handley et al., 1999; Swap et al., 2004), and in several other crop species in particular (Lopes and Araus, 2006; Lopes et al., 2004).
5.6. Implications for historical dietary reconstruction

The implications of this study are not limited to the Andes. Millions of tonnes of guano were exported to Europe and North America during the nineteenth century (Fig. 9). Peruvian seabird guano was the most highly prized fertilizer at the time and a focal point of the Peruvian economy (Cushman, 2003; Hollett, 2008; Mathew, 1972). Between 1851 and 1872, ten million tons of guano were exported from the Chincha Islands group (Fig. 1) alone, the value of which would be equivalent to approximately $13.6 billion 2011 USD (Hollett, 2008). Great Britain and the United States were by far the most significant importers of guano during the nineteenth century, although many other European countries (France, the Netherlands, Italy, Belgium, Norway, Sweden, Russia) were also involved in the trade (Clark and Foster, 2009; Hollett, 2008).

It is very difficult to discern to which crops guano was predominantly applied during the guano boom of the nineteenth century as no records are readily available, but it appears that various grasses, wheat, and potatoes all received significant fertilization with guano in Britain (Mathew, 1970). In the United States, guano was definitely used on corn, wheat, oats, tobacco, and cotton (Jordan, 1950; Taylor, 1947), although it was marketed as a miracle fertilizer that would aid in the growth of any crop (e.g. Tegarden, 1854). It is thus probable that a much wider variety of crops were fertilized with guano.

In recent years, stable isotope analysis of historic human skeletal material has provided considerable insight into the diet and life-ways of these individuals (e.g. Cox and Sealy, 1997; Cox et al., 2001; Katzenberg et al., 2000; Katzenberg, 1995; Klippel, 2001; Roy et al., 2005; Schroeder et al., 2009; Sealy et al., 1995; Valentin et al., 2006). Given the historic importance of guano fertilization, particularly in the United States and Britain, the potential influence of guano on the isotopic composition of foods that humans may have been consuming must be considered.

5.7. Implications for prehispanic Andean use of the guano islands

As discussed previously, direct evidence for the use of seabird guano in the Andes prior to the sixteenth century is lacking. Speculation about the potential use of guano and/or the guano islands has therefore largely been informed by prehispanic ceramic art and material culture, which has focused heavily on the Moche for two reasons. First, guano birds, the guano islands, and related imagery play significant roles in Moche material culture (Fig. 10). Scenes depicting the hunting of seals or sea lions are also common (Fig. 11) and often portray what have been interpreted as guano islands (Fig. 12). Depictions of boats loaded with what have been suggested to be captives destined for the guano islands have also been found (Fig. 13). Second, the vast majority of the objects recovered from deposits on the guano islands during the nineteenth century display Moche stylistic elements (Kubler, 1948), even those from islands off the south coast of Peru (e.g. Chincha). The relatively small number of isotopic data for human bone collagen analyzed thus far from Moche sites are characterized by relatively low δ15N values when compared with human δ15N values from southern Peru (Fig. 14). Given the clear evidence from both growth chamber and field studies for the potentially massive increase in plant tissue δ15N values resulting from guano fertilization, the data recorded thus far for archaeological bone collagen suggest that the Moche were not making use of seabird guano as

Fig. 12. Moche fine line depiction of a sea lion hunt. The object on the right has been interpreted as a guano island. A structure, two individuals, and two sealed containers (usually associated with scenes of ritual practice) can clearly be seen on the island. Drawing by Donna McClelland, courtesy of Don McClelland.

Fig. 13. Moche vessel depicting a boat containing a captive and an individual paddling. This likely represents the transportation of an individual destined to be ritually killed on one of the guano islands (Steve Bourget, Personal Communication). Photograph courtesy of Museo Larco (Catalog #ML003202).
... the potential effects of long term fertilization with both camelid dung and seabird guano on the isotopic composition of plants. Moreover, studies examining the influence of these fertilizers applied in different concentrations, and on different varieties of plants (e.g. leguminous plants), would be useful to better understand how the isotopic composition of plants with variable N-use strategies are affected by these, and other fertilizers. The stable isotope biogeochemistry of additional fertilizers and crops that may have been significant in other parts of the world must also be studied.

The increase in plant $\delta^{15}$N values resulting from camelid dung fertilization has the potential to complicate dietary interpretations in terms of the relative proportions of plant and animal protein in the diet, provided these sources have similar carbon isotopic compositions. The increase in plant $\delta^{15}$N values resulting from seabird guano fertilization of maize has the potential to complicate dietary interpretations in terms of the relative proportions of maize and marine protein in the diet. This must be taken into account in areas where the potential for maize fertilization with seabird guano exists, which includes the Andes, as well as the United States and Europe during the guano boom of the middle to late nineteenth century. The results of this study also demonstrate the inherent difficulties associated with quantitative dietary reconstruction using the bulk isotopic composition of bone collagen or similar tissues. The development and refinement of compound-specific isotopic techniques will likely aid in resolving these and similar issues.

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**Appendix A. Supplementary material**

Supplementary material associated with this article can be found, in the online version, at [http://dx.doi.org/10.1016/j.jas.2012.06.035](http://dx.doi.org/10.1016/j.jas.2012.06.035).

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