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Plant sulfur isotopic compositions are altered by marine fertilizers

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Abstract

This study presents sulfur isotope compositions ($\delta^{34}S$) for plants grown in a series of growth chamber and field experiments under controlled conditions. Maize, beans, and squash fertilized with a marine fertilizer (seabird guano) were significantly enriched in ^{34}S relative to the unfertilized control plants (by +4.0 to +7.2‰) in the growth chamber experiments. No ^{34}S enrichment was detected in the plants from the field experiment, which likely relates to the recent use of ammonium sulfate fertilizer in these fields and the retention of residual sulfate with a comparatively low $\delta^{34}S$. The field experiment provided a basis to estimate the apparent fractionation between soil and plant S associated with uptake and assimilation ($\Delta^{34}S$), which ranged between −4 and −6‰ depending on the taxon and tissue. The use of marine fertilizers has the capacity to increase plant $\delta^{34}S$ values and complicate quantitative reconstructions of ancient diet based on bulk stable isotope data.

Keywords Stable isotopes · Sulfur isotopes · Fertilizers · Paleodiet

Introduction

The analysis of stable isotopes derived from ancient human tissues has provided enormous insight into the evolution of prehistoric human diets. This research has predominantly examined the carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic compositions of bone collagen. Sulfur (S) isotopic compositions ($\delta^{34}S$) have been employed with increasing regularity over the last 10 years to further refine paleodietary interpretations. A key issue that rests largely upon the interpretation of isotopic data is the relative importance of plant and animal protein in the diet. For example, this has been the case when comparing the diets of modern humans and Neanderthals in Europe as well as shifts in diet at the Mesolithic-Neolithic transition (Richards 2002). Nitrogen isotopic compositions tend to increase at fairly regular intervals (+3 to +5‰) at each trophic level (Minagawa and Wada 1984), and because of this, they allow the differentiation of diets based predominantly on plant and animal protein, for example, between herbivores and carnivores (DeNiro and Epstein 1981). A potentially complicating factor in this interpretation is the effects of animal manures on plant nitrogen isotopic compositions. Experimental studies have demonstrated the potential for animal manures to alter the nitrogen isotopic compositions of cultigens in such a way that they more closely resemble the tissues of coeval herbivores, with fertilized plants being enriched in ^{15}N relative to unfertilized plants to varying degrees (reviewed by Szpak 2014). These effects range from modest (< 4‰ for many domestic herbivore manures) to extreme (> 30‰ for seabird guano) (Bogaard et al. 2007; Szpak et al. 2012a). In the absence of supporting evidence for the use of animal fertilizers, such as isotopic analysis of archaeobotanical remains (Bogaard et al. 2013) and geochemical or micromorphological characterization of prehistoric fields (Bull et al. 2001), carbon, and nitrogen isotopic compositions derived from bulk bone collagen lose considerable resolution for the differentiation of

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plant vs. animal protein (Bogaard et al. 2007) or plant vs. marine protein (Szpak et al. 2012b) in the diet. The inclusion of additional isotopic markers in paleodietary assessments is one way to overcome these ambiguities (e.g., Jaouen et al. 2012; Jaouen et al. 2016; McCarthy et al. 2007; Reynard et al. 2010).

Sulfur isotope analysis provides a useful complement to the analysis of $\delta^{13}C$ and $\delta^{15}N$ values. Because sulfur isotopic compositions in biological tissues are controlled primarily by the sulfur isotopic composition of the ultimate sulfate source, they are an indicator of both diet and locality (reviewed by Canfield 2001; Nehlich 2015; Richards et al. 2003). For both terrestrial and freshwater organisms, $\delta^{34}S$ values are highly variable, but on average tend to be higher in freshwater relative to terrestrial environments. Marine organisms have more uniform $\delta^{34}S$ values that are consistently higher than either terrestrial or freshwater organisms. Understanding the factors that can influence the isotopic compositions of potential food sources is a crucial part of paleodietary and paleoecological studies (Casey and Post 2011). With respect to plants, considerable progress has been made in documenting the causes of variation (environmental and anthropogenic) in carbon and nitrogen isotopic compositions (reviewed by Craine et al. 2009; Farquhar et al. 1989; Hobbie and Högberg 2012; Högberg 1997; Szpak 2014). Such studies are of the utmost importance because producers (plants, algae, and cyanobacteria) link biological and geological systems through their uptake of dissolved nutrients. It is at this nutrient-producer level where various environmental parameters most strongly influence isotopic compositions. With respect to sulfur isotopes, however, studies of plants have been limited.

A previous study examining the effects of seabird guano fertilization on plant nitrogen isotopic compositions demonstrated the difficulty in differentiating diets with a high marine food component and diets with a high guano-fertilized maize component (Szpak et al. 2012b). Sulfur isotopes were suggested as a possible means with which to differentiate these two diets. The purpose of this study was to examine the effects of applying a fertilizer of marine origin (seabird guano) to terrestrial plants under controlled conditions to assess whether this would impact plant sulfur isotopic compositions. While a large number of experimental studies have tested the impact of animal and other organic fertilizers on plant or soil nitrogen isotopic compositions (reviewed by Szpak 2014), to the best of our knowledge, none have examined sulfur isotopes. Plant $\delta^{34}S$ values were significantly higher when the marine fertilizer was applied, which has the potential to complicate interpretations of freshwater fish consumption in past human populations.

Sulfur isotopes in plants

Sulfur is essential for growth and various metabolic functions in plants. The primary source of sulfur for most terrestrial plants is dissolved sulfate (SO_4^{2-}), except in cases where soils are well-drained and dissolved SO_4^{2-} is leached by excess water (Yi-Balan et al. 2014). Plants take up SO_4^{2-}, which is then reduced to sulfide (S^{2-}) and assimilated as cysteine (Leustek and Saito 1999). Cysteine is the precursor to methionine as well as a host of other sulfur-containing organic compounds (Tcherkez and Tea 2013). In addition to dissolved S in the soil, plants can assimilate gaseous S species (SO_2 and H_2S) into organic S via foliar uptake, although the relative importance of atmospheric S will vary as a function of soil and atmospheric S availability (Krouse 1977).

A small number of studies have documented that there is relatively little discrimination against ^{34}S with the uptake and assimilation of sulfur in several plant species, with whole plants typically having $\delta^{34}S$ values similar to environmental SO_4^{2-} (Trust and Fry 1992). Although studies examining intraplant variation in $\delta^{34}S$ values are even fewer, a pattern has emerged wherein stems and roots are 1–2‰ depleted of ^{34}S relative to source SO_4^{2-}, while grains and leaves are 1–2‰ enriched (Tcherkez and Tea 2013). Thus, unlike N, which can be taken up and assimilated via several different pathways (Evans 2001; Näsholm et al. 2009; Sparks 2009), resulting in widely varying $\delta^{15}N$ values in plant tissues regardless of the $\delta^{15}N$ of the source N (Szpak 2014), plant $\delta^{34}S$ values appear to more consistently reflect source $\delta^{34}S$ values.

Aerosolized SO_4^{2-} derived from seawater has a similar $\delta^{34}S$ value relative to seawater SO_4^{2-} (+21‰; Rees et al. 1978) and studies have shown that soils, plants, and animals in areas affected by this marine SO_4^{2-} have elevated $\delta^{34}S$ values (Bern et al. 2015; Zazzo et al. 2011). The scale of this "sea spray" effect may be relatively localized or extend considerable distances (> 100 km) inland. The majority of sulfur isotope studies of plants have focused on either (1) assessing the relative importance of marine and terrestrial sulfate in estuarine or salt marsh environments (e.g., Fry et al. 1982; Peterson et al. 1985; Raven and Scrimgeour 1997) or (2) quantifying the importance of anthropogenic S to plant tissues derived from pollution (e.g., Gebauer et al. 1994; Krouse 1977; Zhao et al. 2003). No studies have investigated the potential impact of agricultural fertilizers on plant $\delta^{34}S$ values.

Materials and methods

The results of this study are derived from three different experiments, two of which occurred in a walk-in growth chamber (hereafter Growth Chamber Experiment A and Growth Chamber Experiment B), while the third occurred under controlled conditions in an agricultural field on the north coast of Peru (hereafter Field Experiment).

Growth chamber experiment A (GCE–A)

The first growth chamber experiment used maize (*Zea mays*) grown under controlled conditions at the Biotron Center for Experimental Climate Change Research at The University of Western Ontario (described in detail in Szpak et al. 2012a). The light period was 13 h (185 W fluorescent bulbs) with a temperature of 25 °C. For the dark period (11 h), the temperature was 18 °C. Relative humidity was maintained at 60% throughout. Maize was grown in free-draining (perforated at the base) 18.9-L buckets containing 16 L of Pro-mix® For Containers (75–85% sphagnum moss, 15–25% perlite, and limestone). Three treatments were used in this experiment: C0 (control, no fertilizer applied), G2 (5 g guano/L), and G3 (10 g guano/L). Each treatment consisted of five replicates. Plant tissues analyzed in this study (grains) were sampled at the completion of the experiment, 115 days after planting.

Growth chamber experiment B (GCE–B)

The second growth chamber experiment was conducted in the same growth chamber and at the same time as GCE−A (described in detail in Szpak et al. 2014). The conditions (light period, light intensity, temperature, relative humidity, watering frequency) were the same. Rather than maize, this experiment used common beans (*Phaseolus vulgaris*) and summer squash (*Cucurbita pepo*), which were grown in 2-L containers with the same Pro-mix® substrate as in GCE−A. For each plant species, there were two treatments: C0 (control, no fertilizer applied) and G1 (2.5 g guano/L). Each treatment consisted of four replicates. Plant tissues analyzed in this study (fruits) were sampled at the completion of the experiment, 75 days after planting. Insufficient material was available for two of the four fertilized squash fruits. The seabird guano used in these experiments was the same as that used in GCE−A.

Field experiment (FE)

The field experiment was conducted in an agricultural field in northern Peru between April 28 and October 4, 2010 (described in detail in Szpak et al. 2012b). Maize was grown in four 6 × 6 m plots: C0 (control, no fertilizer applied), AS (fertilized with ammonium sulfate), DU (fertilized with alpaca dung), and SG (fertilized with seabird guano).

Sample preparation

Plant and fertilizer samples from the growth chamber studies were frozen immediately after sampling and then oven dried at 90 °C. Plant and fertilizer samples from the field study were initially air-dried on site and subsequently oven dried at 90 °C. After drying, plant samples were ground to a fine powder using a Wig-L-Bug mechanical shaker and stored at room temperature. Fertilizer samples were ground to a fine powder using an agate mortar and pestle.

Stable isotope and elemental analysis

Sulfur elemental and isotopic compositions were determined with an Isoprime 100 isotope-ratio mass spectrometer coupled to a Vario MICRO cube elemental analyzer (Elementar, Hanau, Germany). Sulfur isotope compositions were calibrated relative to VCDT, using a two-point calibration with IAEA-S-1 (silver sulfide, $\delta^{34}S$ −0.30‰) and NBS-127 (barium sulfate, $\delta^{34}S$ +20.3‰). Accuracy and precision were monitored using the following standard reference materials: methionine ($\delta^{34}S$ +9.1 ± 0.6‰), NIST 1577c (bovine liver; $\delta^{34}S$ +1.7 ± 0.5‰), casein protein ($\delta^{34}S$ +6.3 ± 0.6‰), SRM-4 (gluten; $\delta^{34}S$ −6.3 ± 0.2‰), SRM-5 (red lentil flour; $\delta^{34}S$ +6.6 ± 0.5‰), and either IAEA-S-3 (silver sulfide, long-term average $\delta^{34}S$ −31.9 ± 0.6‰) or IAEA-SO-5 (barium sulfate, long-term average $\delta^{34}S$ +0.7 ± 0.3‰). Further details for and results of the calibration and check standards in each analytical session can be found in the Supplementary Information.

Elemental compositions were calibrated post-run by applying a correction factor calculated using the known sulfur contents %S compositions of IAEA-S-1 (12.9 wt%), IAEA-S-3 (12.9 wt%), IAEA-SO-5 (13.7 wt%), and NBS-127 (13.7 wt%) together with the mass of each standard and the amplitude of its SO_2 beam in the IRMS. Twenty-two out of 104 samples were analyzed in duplicate and the mean difference between replicates was 0.4‰ for $\delta^{34}S$. The overall analytical uncertainty for $\delta^{34}S$ measurements was determined to be ± 0.7‰ (see Supplementary Material).

Data treatment

The contribution of guano-derived sulfur to bulk maize organs (GCE−A) was determined using a two-source Bayesian mixing model in the SIAR package (Parnell et al. 2010) in R 3.0.3 for Mac OS X (R Development Core Team 2007). The sources were seabird guano, with a $\delta^{34}S$ value determined by direct analysis of the fertilizer, and endogenous substrate sulfur, with a $\delta^{34}S$ value determined by applying an adjustment for bulk apparent fractionation ($\Delta^{34}S$) between maize grains and source S. This $\Delta^{34}S$ value was calculated by comparing the average $\delta^{34}S$ value of the field-grown maize fertilized with ammonium sulfate to the $\delta^{34}S$ value of the ammonium sulfate. The apparent fractionations ($\Delta^{34}S$) for beans and squash were estimated by applying the $\Delta^{34}S$ for maize calculated from the field study to the GCE−A maize to estimate the $\delta^{34}S$ of the substrate S used in both GCE−A and GCE−B. Thus, substrate $\delta^{34}S$ value was then used to derive a $\Delta^{34}S$ value for the control (unfertilized) beans and squash grown in GCE−B.

Results and discussion

Relationship between plant and source $\delta^{34}S$ values

The sulfur isotopic and elemental compositions for the fertilizers used in both the growth chamber and field experiments are presented in Table 1. To determine the relative contribution of fertilizer-derived sulfur to plant tissues, it was necessary to estimate the apparent fractionation ($\Delta^{34}S$) between source and bulk plant organ $\delta^{34}S$ values. In the field experiment, one of the treatments utilized ammonium sulfate fertilizer, with 24 wt% sulfur and a $\delta^{34}S$ of +6.1‰. This was the same fertilizer that had been used in the field prior to the experiment. Given the very low quantities of mineralized S available in soils (< 5% of total soil S) (Scherer 2009), it is reasonable to assume that the ammonium sulfate was the only quantitatively significant S source in this plot. Using the average foliar and grain $\delta^{34}S$ values resulted in $\Delta^{34}S$ of −4.3 ± 1.2‰ for leaves and −5.2 ± 0.3‰ for grains. These values are lower than the often cited −1 to 0‰ range for $\Delta^{34}S$ of whole plant bulk S relative to source S (Tcherkez and Tea 2013); it is important to remember, however, that these values (−1 to 0‰) are based on limited experimental data. Tanz and Schmidt (2010) reported a $\Delta^{34}S$ value of −5.0‰ for leek (*Allium porrum*), which is comparable to the value calculated for maize in this study.

The $\delta^{34}S$ values of the unfertilized maize grains and bean fruits grown in the growth chamber experiment were +3.9 ± 0.8‰ and +4.1 ± 1.8‰, respectively. On the basis of the estimated $\Delta^{34}S$ values for maize grains (determined using the ammonium sulfate fertilized plants from the field experiment), this suggests a $\delta^{34}S$ value of +9.1‰ for the control substrate S. Using this value for the source S suggests an apparent fractionation ($\Delta^{34}S$) of −5.0 ± 1.8‰ for bean fruits and −5.7 ± 1.6‰ for squash fruits.

Growth chamber experiments

Sulfur isotopic and elemental compositions for the maize (GCE−A), beans and squash (GCE−B) from the two growth chamber experiments are presented in Table 2 and Fig. 1. Guano-fertilized maize was characterized by significantly higher $\delta^{34}S$ relative to control plants (unpaired *t* test) for both the G2 ($t_{[8]} = 15.3$, $p < 0.001$) and G3 ($t_{[8]} = 13.5$, $p < 0.001$) treatments. The maize that received the highest amount of guano (G3, 10 g guano/L) had a $\delta^{34}S$ value that was slightly lower than the maize that received half as much guano (G2, 5 g guano/L), but the difference was not significant ($p = 0.30$). Overall, there was a positive correlation between the $\delta^{15}N$ and $\delta^{34}S$ values for all of the growth chamber plants (Spearman's ρ, $\rho = 0.80$, $p < 0.001$), demonstrating the uptake of both nitrogen and sulfur derived from the guano fertilizer (Fig. 2).

The guano-fertilized maize was characterized by the highest $\delta^{34}S$ values, which were +7.2‰ (G2) and +6.7‰ (G3) higher than the control plants. Using the two-source Bayesian mixing model, the amount of plant S derived from guano was 93% (mean, 95% credibility interval 81–100%) for the G2 maize and 92% (mean, 95% credibility 73–100%) for the G3 maize. These results suggests that the amounts of guano-derived S available in the G2 and G3 treatments were very similar and most likely far exceeded plant demand, which is relatively small. The amount of sulfur present in the guano fertilizer (2.8 wt% S) is orders of magnitude greater than the total S content of most soils (Scherer 2009). Therefore, no additional ^{34}S enrichment would be expected at higher fertilizer application rates.

The guano-fertilized beans and squash (G1, 2.5 g guano/L) had $\delta^{34}S$ values that were higher than the unfertilized control plants by 5.1 (for beans) and 5.7‰ (for squash). Using the two-source Bayesian mixing model, the amount of plant S derived from guano was 66% (mean, 95% credibility interval 41–96%) for the guano-fertilized beans. The small sample size for the fertilized squash precludes the possibility of making quantitative statements about the relative amount of S derived from the guano. Nonetheless, the results demonstrate the uptake of a substantial amount of sulfur derived from the seabird guano, likely similar in magnitude to the beans (Fig. 1).

Field experiment

Sulfur isotopic and elemental compositions for the maize from the field experiment are presented in Table 3 and Fig. 3. The maize grains were depleted of ^{34}S relative to the leaves for each of the treatments (−1.6‰ for C0, −0.3 for AS, −2.1 for DU, −2.1 for SG), a pattern that is consistent with results

Table 1 Sulfur isotopic and elemental compositions for fertilizers used in the growth chamber and field experiments

Lab ID	Type	Experiment	$\delta^{34}S$	wt% S	$\delta^{15}N$[a]	wt% N[a]
9431	Seabird guano	Growth chamber	+15.4[b]	2.79[b]	+26.7	11.20
9428	Alpaca dung	Field	+6.7[b]	0.55[b]	+14.0	2.64
9430	Ammonium sulfate	Field	+6.1	23.76	0.0	20.91
9429	Seabird guano	Field	+16.5[b]	4.29[b]	+39.1	6.71

[a] Nitrogen isotope and elemental data are from Szpak et al. (2012a, b)

[b] Mean of two analyses

Table 2 Sulfur isotopic and elemental compositions for plants grown in the growth chamber experiments

Lab ID	Species	Organ	Guano (g/L soil)	$\delta^{34}S$	$\delta^{15}N$[a]	%S
7450	Maize	Grain	0	+2.6	+2.0	0.18
7461	Maize	Grain	0	+3.9	+2.3	0.19
7462	Maize	Grain	0	+4.3	+2.4	0.20
7463	Maize	Grain	0	+3.9	+3.1	0.20
7464	Maize	Grain	0	+4.7	+3.6	0.19
			Mean	+3.9 ± 0.8	+2.7 ± 0.7	0.19 ± 0.01
7452	Maize	Grain	5	+10.2	+26.7	0.15
7469	Maize	Grain	5	+10.5	+22.8	0.16
7470	Maize	Grain	5	+11.8	+26.8	0.19
7471	Maize	Grain	5	+11.3	+26.1	0.15
7472	Maize	Grain	5	+11.6	+25.3	0.17
			Mean	+11.1 ± 0.7	+25.5 ± 1.6	0.16 ± 0.02
7451	Maize	Grain	10	+10.3	+36.7	0.19
7465	Maize	Grain	10	+9.9	+34.2	0.12
7466	Maize	Grain	10	+10.4	+33.7	0.15
7467	Maize	Grain	10	+10.3	+29.1	0.12
7468	Maize	Grain	10	+11.9	+31.9	0.12
			Mean	+10.6 ± 0.8	+33.1 ± 2.8	0.14 ± 0.03
7473	Squash	Fruit	0	+4.4	+0.1	0.28
7474	Squash	Fruit	0	+1.8	+0.3	0.27
7475	Squash	Fruit	0	+5.1	+0.3	0.32
7476	Squash	Fruit	0	+2.3	+0.2	0.35
			Mean	+3.4 ± 1.6	+0.2 ± 0.1	0.31 ± 0.04
7477	Squash	Fruit	2.5	+8.0	+23.2	0.26
7478	Squash	Fruit	2.5	+10.1	+27.0	0.33
			Mean	+9.1 ± 1.5	+25.1 ± 2.7	0.30 ± 0.05
7449	Bean	Fruit	0	+1.5	+0.3	0.23
7482	Bean	Fruit	0	+4.5	+0.8	0.34
7483	Bean	Fruit	0	+5.1	−0.4	0.34
7484	Bean	Fruit	0	+5.4	−0.8	0.27
			Mean	+4.1 ± 1.8	0.0 ± 0.7	0.30 ± 0.05
7448	Bean	Fruit	2.5	+8.5	+16.4	0.34
7479	Bean	Fruit	2.5	+9.8	+18.1	0.36
7480	Bean	Fruit	2.5	+9.2	+16.5	0.37
7481	Bean	Fruit	2.5	+8.5	+13.9	0.44
			Mean	+9.0 ± 0.6	+16.2 ± 1.7	0.38 ± 0.04

[a] Nitrogen isotope data are from Szpak et al. (2012a, 2014)

obtained from wheat (*Tricitum aestivum*), the only other plant species where intraplant $\delta^{34}S$ variation has been studied (summarized in Tcherkez and Tea 2013). Unlike the growth chamber experiments, however, where there were clear distinctions between guano-fertilized and control plants, there were no differences between the control and any of the fertilized treatments (seabird guano, alpaca dung, ammonium sulfate) in the field experiment. This is not surprising for the alpaca dung and ammonium sulfate treatments because the fertilizer $\delta^{34}S$ values were nearly identical (Table 1). At the earliest sampling dates, the guano-fertilized maize did have slightly higher $\delta^{34}S$ values than the maize in the three other plots, but the difference was small and was not maintained past the 60-day mark (Fig. 3). Unlike the growth chamber plants, which were characterized by a strong correlation between plant $\delta^{15}N$ and $\delta^{34}S$ values (Fig. 2), there was no correlation between plant $\delta^{15}N$ and $\delta^{34}S$ for the field-grown maize (Spearman's ρ, $\rho = -0.22$, $p = 0.06$, Fig. 4). The $\delta^{34}S$ values for the field-grown maize were relatively low, particularly in the grains harvested at the end of the experiment, which ranged between −2 and +1‰ for all treatments.

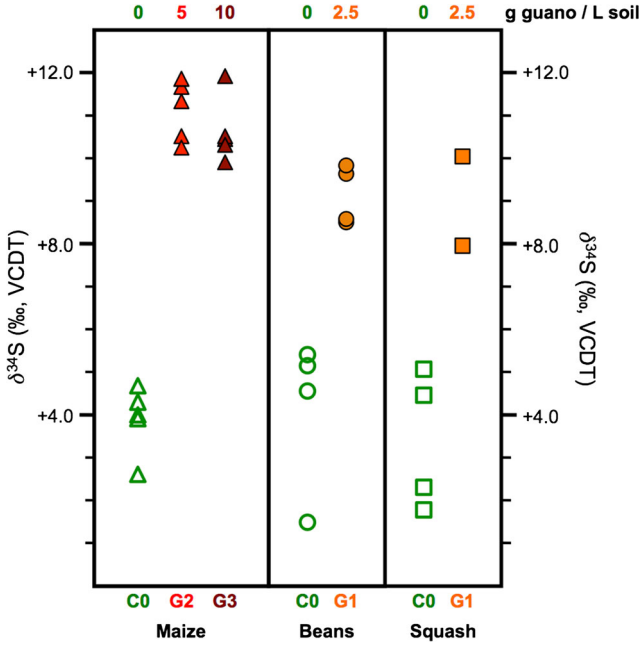

Fig. 1 $\delta^{34}S$ values for plants grown in the two growth chamber experiments. Open symbols indicate unfertilized control (C0) plants and shaded symbols indicate guano-fertilized plants (G1, G2, G3). The amount of guano applied in each treatment is indicated at the top of the plot

Because the seabird guano fertilizer had a very distinct $\delta^{34}S$ value from the other fertilizers, the guano-fertilized plants were clearly acquiring the bulk of their sulfur from another source, which was common to all four plots. Prior to the experiment, ammonium sulfate was regularly applied to the field where the experimental plots were located. Mizota and Sasaki (1996) have demonstrated that the long-term application of fertilizers with high sulfur contents has the capacity to alter the sulfur isotopic compositions of soils, such that they

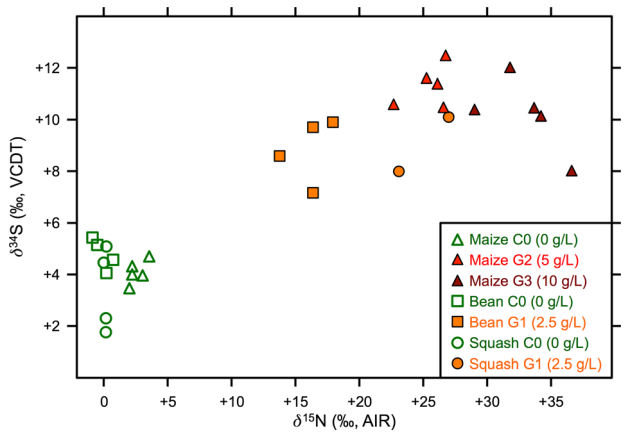

Fig. 2 Bivariate plot of $\delta^{34}S$ and $\delta^{15}N$ values for all plants from the two growth chamber experiments: maize (triangles), beans (rectangles), and squash (circles). Plants that were not fertilized are indicated by open symbols and plants that were fertilized with guano are indicated by filled symbols. The legend indicates the amount of fertilizer applied (in g guano/L soil) for each of the treatments

Table 3 Sulfur isotopic and elemental compositions for plants grown in the field experiment

Lab ID	Organ	Treatment[a]	Date[b]	$\delta^{34}S$	$\delta^{15}N$[c]	%S
9438	Leaf	DU	18	+1.7	+7.2	0.39
9439	Leaf	DU	18	+1.4	+10.6	0.40
9440	Leaf	DU	18	+0.9	+11.3	0.37
7459	Leaf	DU	31	+3.3	+3.8	0.49
7487	Leaf	DU	46	+0.7	+7.8	0.36
7491	Leaf	DU	61	+1.4	+6.7	0.43
9450	Leaf	DU	76	+3.0	+8.1	0.60
9451	Leaf	DU	76	−1.1	+8.2	0.26
9452	Leaf	DU	76	+2.3	+5.5	0.44
7495	Leaf	DU	91	+1.4	+16.1	0.30
9470	Leaf	DU	107	+2.4	+14.3	0.29
9474	Leaf	DU	122	+2.5	+7.2	0.29
9462	Leaf	DU	138	+1.1	+6.2	0.42
9463	Leaf	DU	138	−0.1	+6.2	0.33
9464	Leaf	DU	138	+1.0	+13.3	0.22
7455	Grain	DU	157	−0.4	+7.8	0.09
9480	Grain	DU	157	−1.7	+7.7	0.11
9481	Grain	DU	157	−2.4	+7.6	0.11
9432	Leaf	AS	18	+2.4	+4.9	0.36
9433	Leaf	AS	18	+3.7	+4.4	0.48
9434	Leaf	AS	18	+2.5	+6.0	0.45
7457	Leaf	AS	31	+2.9	+0.3	0.33
7485	Leaf	AS	46	+1.2	+7.4	0.34
7489	Leaf	AS	61	+2.0	+4.6	0.24
9444	Leaf	AS	76	+1.4	+1.7	0.29
9445	Leaf	AS	76	+0.3	+8.1	0.27
9446	Leaf	AS	76	+1.7	+2.7	0.28
7493	Leaf	AS	91	+2.2	+2.8	0.30
9468	Leaf	AS	107	+2.7	+1.1	0.34
9472	Leaf	AS	122	+0.6	+3.9	0.23
9456	Leaf	AS	138	−0.3	+5.9	0.23
9457	Leaf	AS	138	+0.4	+5.7	0.26
9458	Leaf	AS	138	+3.4	+3.6	0.38
7453	Grain	AS	157	+0.7	+5.7	0.10
9476	Grain	AS	157	+0.6	+5.6	0.13
9477	Grain	AS	157	+1.2	+5.9	0.11
9435	Leaf	C0	18	+3.7	+8.0	0.43
9436	Leaf	C0	18	+3.1	+5.0	0.51
9437	Leaf	C0	18	+3.7	+4.8	0.43
7458	Leaf	C0	31	+2.6	+6.2	0.26
7486	Leaf	C0	46	+2.4	+5.4	0.36
7490	Leaf	C0	61	+1.7	+3.1	0.34
9447	Leaf	C0	76	+1.5	+3.6	0.31
9448	Leaf	C0	76	+1.8	+5.4	0.27
9449	Leaf	C0	76	+2.6	+4.7	0.35
7494	Leaf	C0	91	+3.3	+3.5	0.34
9469	Leaf	C0	107	+2.2	+4.0	0.31
9473	Leaf	C0	122	+2.5	+2.5	0.32

Table 3 (continued)

Lab ID	Organ	Treatment[a]	Date[b]	$\delta^{34}S$	$\delta^{15}N$[c]	%S
9459	Leaf	C0	138	+1.1	+2.1	0.27
9460	Leaf	C0	138	+0.9	+4.1	0.24
9461	Leaf	C0	138	+3.3	+4.4	0.32
7454	Grain	C0	157	+0.7	+6.1	0.13
9478	Grain	C0	157	+0.4	+6.1	0.12
9479	Grain	C0	157	−0.7	+6.0	0.12
9441	Leaf	SG	18	+6.4	+12.5	0.47
9442	Leaf	SG	18	+2.5	+10.6	0.37
9443	Leaf	SG	18	+4.3	+14.7	0.45
7460	Leaf	SG	31	+4.8	+22.9	0.40
7488	Leaf	SG	46	+3.1	+38.3	0.24
7492	Leaf	SG	61	+2.0	+30.4	0.32
9453	Leaf	SG	76	+1.6	+33.3	0.28
9454	Leaf	SG	76	−0.6	+31.3	0.23
9455	Leaf	SG	76	+1.2	+25.8	0.23
7496	Leaf	SG	91	+1.2	+26.8	0.29
9471	Leaf	SG	107	+1.8	+32.2	0.12
9475	Leaf	SG	122	+3.2	+29.3	0.27
9465	Leaf	SG	138	+0.4	+20.8	0.23
9466	Leaf	SG	138	+0.2	+23.4	0.28
9467	Leaf	SG	138	+2.2	+25.9	0.39
7456	Grain	SG	157	−0.2	+21.4	0.11
9482	Grain	SG	157	−2.7	+21.2	0.11
9483	Grain	SG	157	−0.5	+21.1	0.10

[a] Treatments: DU (alpaca dung), AS (ammonium sulfate), C0 (control, unfertilized), SG (seabird guano)

[b] Date of sampling in number of days after planting

[c] Data are from Szpak et al. (2012b)

resemble that of the fertilizer. The high sulfur content of ammonium sulfate (24 wt%) and its comparatively low $\delta^{34}S$ value (+6.1‰) are consistent with this explanation. The results of the field study do not, therefore, provide any data that are useful with respect to paleodietary studies but serve as a caution for future studies examining similar questions. The sulfur isotope compositions of crops grown in fields, such as this, are more likely to be overwhelmed by modern fertilizer applications because of the relatively low abundance of sulfur in the soil and the extremely high abundance in ammonium sulfate—similar problems should not be encountered if fertilizers lacking sulfur were used (e.g., ammonium nitrate, potassium nitrate, or urea).

Paleodietary implications

The results of this study demonstrate that the use of an organic fertilizer of marine origin can significantly alter the $\delta^{34}S$ values of plants. There are, however, several caveats to this general conclusion. First, the extent to which a marine fertilizer might impact plant $\delta^{34}S$ values will be dependent on the sulfur isotope compositions of biogenic soil sulfur, its abundance and availability. In some environments, such as New Zealand and Ireland, large areas of land are characterized by soil $\delta^{34}S$ values that are quite high, suggesting a widespread sea spray effect (Kusakabe et al. 1976; Zazzo et al. 2011). In situations such as those, the application of a marine fertilizer might have a much less significant effect (or no discernable effect at all) on plant $\delta^{34}S$ values. Second, the marine fertilizer must contain an appreciable quantity of sulfur. An "appreciable quantity" will vary depending on the availability of endogenous sulfur in the soil as well as the amount of aerosolized

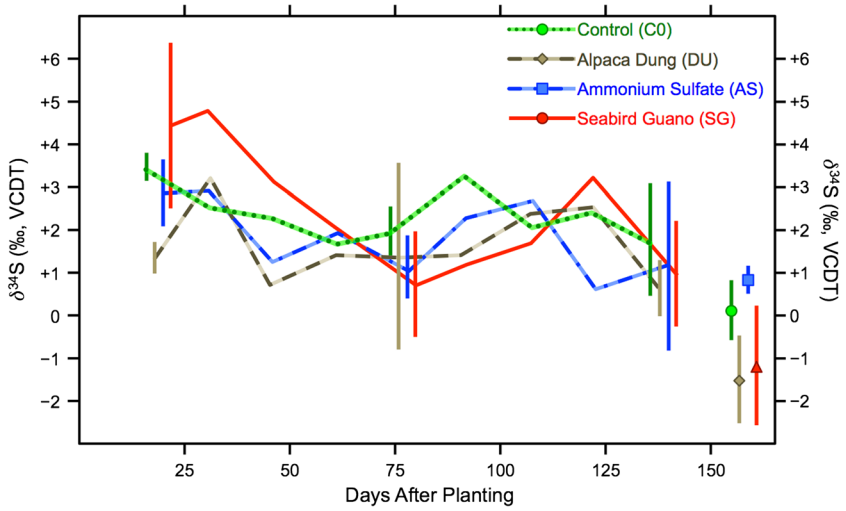

Fig. 3 Sulfur isotope compositions for maize leaves sampled at bimonthly intervals (points between 18 and 138 days after planting) and maize grains sampled at the end of the experiment (points at 157 days). Data for leaves at 18, 76, and 138 days are the mean and standard deviation of three different leaves, while all other points are for single leaves. Data for grains are the mean of grains sampled from three different cobs. The data points for the four different treatments at 18, 76, 138, and 157 days have been slightly offset along the *x*-axis for the sake of readability but were sampled on the same day

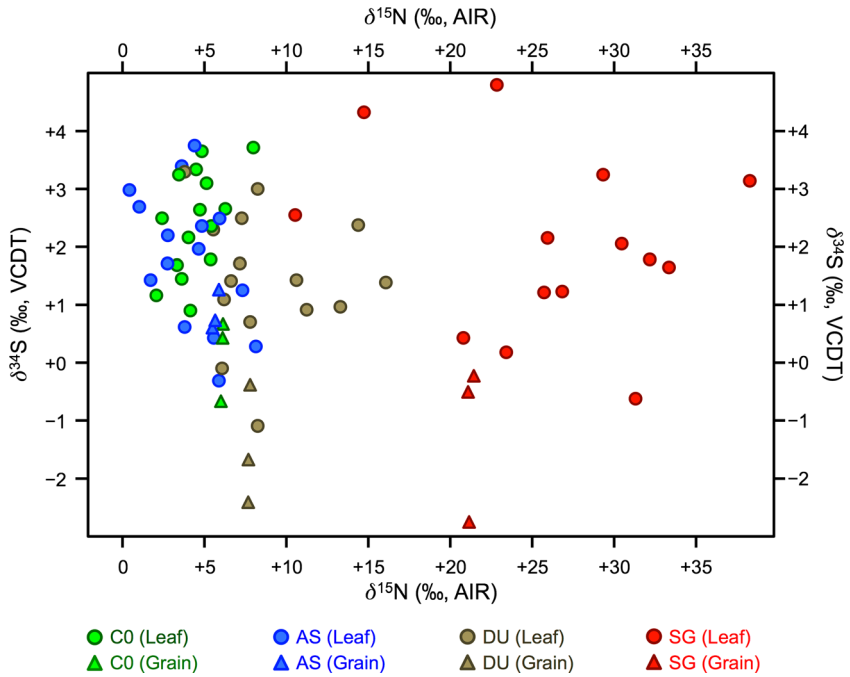

Fig. 4 Bivariate plot of $\delta^{34}S$ and $\delta^{15}N$ values for maize from the field experiment: leaves (circles) and grains (triangles)

sulfur available for plants, although the latter of these is unlikely to have been quantitatively significant prior to industrialization. As the amount of sulfur available to plants from non-fertilizer sources increases, the amount of sulfur absorbed and assimilated from fertilizers will necessarily decrease. Since excessive concentrations of sulfur in plant tissues can be toxic (Rennenberg 1984), plants will not absorb excess sulfur indefinitely and instead, regulatory mechanisms will limit uptake and assimilation (Hawkesford and De Kok 2006). In general then, for plant $\delta^{34}S$ values to be impacted by marine fertilizers, the sulfur isotope composition of the fertilizer must be significantly different than that of the endogenous sulfur sources (soil *and* atmospheric) and the amount of sulfur from the fertilizer must not be completely overwhelmed by endogenous sources.

The seabird guanos used in this study had $\delta^{34}S$ values of +15.4‰ (growth chamber) and +16.5‰ (field). Given that marine nutrients, plants, and animals tend have consistently high and relatively similar $\delta^{34}S$ values regardless of trophic level, other marine fertilizers should have similarly high $\delta^{34}S$ values (Fry 1988; Gaston et al. 2004; Ostrom and Fry 1993). Thus, while the extremely high nitrogen isotopic and elemental compositions of seabird guano translate to higher plant $\delta^{15}N$ values than would be observed with any other marine fertilizer (Szpak et al. 2012a), the more homogenous $\delta^{34}S$ values that should occur in other marine fertilizers suggest that the results presented in this study are not anomalous.

Aside from seabird guano, which was of great economic importance globally in the nineteenth century (Cushman 2013) and was likely a significant fertilizer prehistorically on the west coast of South America (Julien 1985), a number of other marine fertilizers may have been used in the past. Generally, direct evidence for the use of these fertilizers is limited and instead relies on documentary or ethnohistoric records. Based on these sources, the following marine fertilizers were used in antiquity: fish heads; offal or entire fish (Ceci 1975; Rowe 1969); marine algae, such as kelp or seaweed (Davidson et al. 1986; Entwistle et al. 2000; Simpson 1985); and bone meal from large marine mammals, such as whales (Reese 2005; Takahashi et al. 1989). An additional source of marine sulfur delivered to agricultural plants may have come from manures derived from domestic mammals provisioned with marine foods. Numerous examples of these practices have been interpreted on the basis of faunal isotopic data for pigs (e.g., Guiry et al. 2012), cattle (e.g., Müldner et al. 2014), and sheep (e.g., Balasse et al. 2009). The potential, therefore, exists for agricultural plants to be systematically enriched in ^{34}S because of marine fertilization in a number of different contexts.

The application of marine fertilizers makes the quantification of protein derived from marine or freshwater sources (both of which tend to have higher $\delta^{34}S$ values than terrestrial plants and animals on average) problematic. The differentiation of protein derived from marine sources and marine-fertilized plants should still be straightforward, except in cases where the plants use the C_4 photosynthetic pathway. Under normal conditions, C_4 plants and marine foods both have high $\delta^{13}C$ values but distinct $\delta^{15}N$ and $\delta^{34}S$ values. When a marine fertilizer is used, however, these distinctions are obscured and this differentiation is no longer straightforward (Szpak et al. 2012b). With respect to C_3 plants, the use of marine fertilizers presents a more significant problem in terms of quantifying

the contribution of freshwater fish. Freshwater fish have $\delta^{13}C$ values that approach or exceed the range of $\delta^{13}C$ values observed in terrestrial plants (Katzenberg et al. 2009; van der Merwe et al. 2003; Vander Zanden and Rasmussen 1999), but $\delta^{34}S$ values that are typically higher than terrestrial plants (Nehlich 2015). Sulfur isotope studies have therefore been used specifically to address freshwater fish consumption in the past. When relatively high $\delta^{34}S$ values are observed in prehistoric human populations, particularly in conjunction with relatively high $\delta^{15}N$ values, this may be interpreted as indicative of diets rich in freshwater fish (Bollongino et al. 2013; Nehlich et al. 2010; Nehlich et al. 2011). Because a fertilizer of marine origin would have the effect of increasing both the $\delta^{15}N$ and $\delta^{34}S$ values (but not the $\delta^{13}C$ values) of plants, the potential exists for the consumption of C_3 plants fertilized with a marine fertilizer to be confused with the consumption of freshwater fish. That said, significant amounts of freshwater fish consumption have typically been interpreted for inland populations, and the ease of access to marine products for these groups must be considered.

Summary and conclusion

Plants are depleted of ^{34}S relative to the sulfur source by 4–6‰. The application of a marine fertilizer can increase the $\delta^{34}S$ values of agricultural plants and in turn humans or animals consuming these plants. This creates a complication in the quantification of different foods to the diet: C_4 plants fertilized with marine fertilizer vs. marine foods and C_3 plants fertilized with marine fertilizer vs. freshwater fish. Even if $\delta^{34}S$ values can be reliably measured from archaeobotanical remains, it would be difficult if not impossible to differentiate between the passive distribution of oceanic sulfate into coastal agricultural fields via sea spray and the active distribution of marine fertilizers. Therefore, this type of fertilization does not lend itself to specific detection via traditional isotopic techniques (e.g., Bogaard et al. 2013), which further underscores the need to expand the scope of paleodietary analyses beyond two or three bulk isotope systems. The possibility of the application of marine fertilizers adds further uncertainty to the quantitative interpretation of isotopic data in paleodietary studies.

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References

Balasse M, Mainland I, Richards MP (2009) Stable isotope evidence for seasonal consumption of marine seaweed by modern and archaeological sheep in the Orkney archipelago (Scotland). Environ Archaeol 14:1–14. https://doi.org/10.1179/174963109X400637

Bern CR, Chadwick OA, Kendall C, Pribil MJ (2015) Steep spatial gradients of volcanic and marine sulfur in Hawaiian rainfall and ecosystems. Sci Total Environ 514:250–260. https://doi.org/10.1016/j.scitotenv.2015.02.001

Bogaard A, Fraser R, Heaton THE, Wallace M, Vaiglova P, Charles M, Jones G, Evershed RP, Styring AK, Andersen NH, Arbogast R-M, Bartosiewicz L, Gardeisen A, Kanstrup M, Maier U, Marinova E, Ninov L, Schäfer M, Stephan E (2013) Crop manuring and intensive land management by Europe's first farmers. Proc Natl Acad Sci U S A 110:12589–12594. https://doi.org/10.1073/pnas.1305918110

Bogaard A, Heaton THE, Poulton P, Merbach I (2007) The impact of manuring on nitrogen isotope ratios in cereals: archaeological implications for reconstruction of diet and crop management practices. J Archaeol Sci 34:335–343. https://doi.org/10.1016/j.jas.2006.04.009

Bollongino R, Nehlich O, Richards MP, Orschiedt J, Thomas MG, Sell C, Fajkošová Z, Powell A, Burger J (2013) 2000 years of parallel societies in Stone Age Central Europe. Science 342:479–481. https://doi.org/10.1126/science.1245049

Bull ID, Evershed RP, Betancourt PP (2001) An organic geochemical investigation of the practice of manuring at a Minoan site on Pseira Island, Crete. Geoarchaeology 16:223–242. https://doi.org/10.1002/1520-6548(200102)16:2<223::AID-GEA1002>3.0.CO;2-7

Canfield DE (2001) Biogeochemistry of sulfur isotopes. Rev Mineral Geochem 43:607–636. https://doi.org/10.2138/gsrmg.43.1.607

Casey MM, Post DM (2011) The problem of isotopic baseline: reconstructing the diet and trophic position of fossil animals. Earth Sci Rev 106:131–148. https://doi.org/10.1016/j.earscirev.2011.02.001

Ceci L (1975) Fish fertilizer: a native North American practice? Science 188:26–30. https://doi.org/10.2307/1740002

Craine JM, Elmore AJ, Aidar MPM, Bustamante M, Dawson TE, Hobbie EA, Kahmen A, Mack MC, McLauchlan KK, Michelsen A, Nardoto GB, Pardo LH, Peñuelas J, Reich PB, Schuur EAG, Stock WD, Templer PH, Virginia RA, Welker JM, Wright IJ (2009) Global patterns of foliar nitrogen isotopes and their relationships with climate, mycorrhizal fungi, foliar nutrient concentrations, and nitrogen availability. New Phytol 183:980–992. https://doi.org/10.1111/j.1469-8137.2009.02917.x

Cushman GT (2013) Guano and the opening of the Pacific World: a global ecological history. Cambridge University Press, Cambridge

Davidson DA, Harkness DD, Simpson IA (1986) The formation of farm mounds on the island of Sanday, Orkney. Geoarchaeology 1:45–59. https://doi.org/10.1002/gea.3340010106

DeNiro MJ, Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals. Geochim Cosmochim Acta 45:341–351. https://doi.org/10.1016/0016-7037(81)90244-1

Entwistle JA, Dodgshon RA, Abrahams PW (2000) An investigation of former land-use activity through the physical and chemical analysis of soils from the Isle of Lewis, Outer Hebrides. Archaeol Prospect 7:171–188. https://doi.org/10.1002/1099-0763(200009)7:3<171::AID-ARP142>3.0.CO;2-G

Evans RD (2001) Physiological mechanisms influencing plant nitrogen isotope composition. Trends Plant Sci 6:121–126. https://doi.org/10.1016/S1360-1385(01)01889-1

Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon isotope discrimination and photosynthesis. Annu Rev Plant Physiol Plant Mol Biol 40:503–537. https://doi.org/10.1146/annurev.pp.40.060189.002443

Fry B (1988) Food web structure on Georges Bank from stable C, N, and S isotopic compositions. Limnol Oceanogr 33:1182–1190. https://doi.org/10.4319/lo.1988.33.5.1182

Fry B, Scalan RS, Winters JK, Parker PL (1982) Sulphur uptake by salt grasses, mangroves, and seagrasses in anaerobic sediments. Geochim Cosmochim Acta 46:1121–1124. https://doi.org/10.1016/0016-7037(82)90063-1

Gaston TF, Kostoglidis A, Suthers IM (2004) The ^{13}C, ^{15}N and ^{34}S signatures of a rocky reef planktivorous fish indicate different coastal discharges of sewage. Mar Freshw Res 55:689–699. https://doi.org/10.1071/mf03142

Gebauer G, Giesemann A, Schulze E, Jäger H (1994) Isotope ratios and concentrations of sulfur and nitrogen in needles and soils of *Picea abies* stands as influenced by atmospheric deposition of sulfur and nitrogen compounds. Plant Soil 164:267–281. https://doi.org/10.1007/bf00010079

Guiry EJ, Noël S, Tourigny E, Grimes V (2012) A stable isotope method for identifying transatlantic origin of pig (*Sus scrofa*) remains at French and English fishing stations in Newfoundland. J Archaeol Sci 39:2012–2022. https://doi.org/10.1016/j.jas.2012.03.004

Hawkesford MJ, De Kok LJ (2006) Managing sulphur metabolism in plants. Plant Cell Environ 29:382–395. https://doi.org/10.1111/j.1365-3040.2005.01470.x

Hobbie EA, Högberg P (2012) Nitrogen isotopes link mycorrhizal fungi and plants to nitrogen dynamics. New Phytol 196:367–382. https://doi.org/10.1111/j.1469-8137.2012.04300.x

Högberg P (1997) Tansley Review No. 95. ^{15}N natural abundance in soil-plant systems. New Phytol 137:179–203. https://doi.org/10.1046/j.1469-8137.1997.00808.x

Jaouen K, Balter V, Herrscher E, Lamboux A, Telouk P, Albarède F (2012) Fe and Cu stable isotopes in archeological human bones and their relationship to sex. Am J Phys Anthropol 148:334–340. https://doi.org/10.1002/ajpa.22053

Jaouen K, Szpak P, Richards MP (2016) Zinc isotope ratios as indicators of diet and trophic level in arctic marine mammals. PLoS One 11: e0152299. https://doi.org/10.1371/journal.pone.0152299

Julien CJ (1985) Guano and resource control in sixteenth-century Arequipa. In: Masuda S, Shimada I, Morris C (eds) Andean ecology and civilization: an interdisciplinary perspective on Andean ecological complementarity. University of Tokyo Press, Tokyo, pp 185–231

Katzenberg MA, Goriunova O, Weber A (2009) Paleodiet reconstruction of Bronze age Siberians from the mortuary site of Khuzhir-Nuge XIV, Lake Baikal. J Archaeol Sci 36:663–674. https://doi.org/10.1016/j.jas.2008.10.012

Krouse HR (1977) Sulphur isotope abundance elucidate uptake of atmospheric sulphur emissions by vegetation. Nature 265:45–46. https://doi.org/10.1038/265045a0

Kusakabe M, Rafter TA, Stout JD, Collie TW (1976) Sulphur isotopic variations in nature. N Z J Sci 19:433–440

Leustek T, Saito K (1999) Sulfate transport and assimilation in plants. Plant Physiol 120:637–644. https://doi.org/10.1104/pp.120.3.637

McCarthy MD, Benner R, Lee C, Fogel ML (2007) Amino acid nitrogen isotopic fractionation patterns as indicators of heterotrophy in plankton, particulate, and dissolved organic matter. Geochim Cosmochim Acta 71:4727–4744. https://doi.org/10.1016/j.gca.2007.06.061

Minagawa M, Wada E (1984) Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between δ^{15}N and animal age. Geochim Cosmochim Acta 48:1135–1140. https://doi.org/10.1016/0016-7037(84)90204-7

Mizota C, Sasaki A (1996) Sulfur isotope composition of soils and fertilizers: differences between Northern and Southern hemispheres. Geoderma 71:77–93. https://doi.org/10.1016/0016-7061(95)00091-7

Müldner G, Britton K, Ervynck A (2014) Inferring animal husbandry strategies in coastal zones through stable isotope analysis: new evidence from the Flemish coastal plain (Belgium, 1st–15th century AD). J Archaeol Sci 41:322–332. https://doi.org/10.1016/j.jas.2013.08.010

Näsholm T, Kielland K, Ganeteg U (2009) Uptake of organic nitrogen by plants. New Phytol 182:31–48. https://doi.org/10.1111/j.1469-8137.2008.02751.x

Nehlich O (2015) The application of sulphur isotope analyses in archaeological research: a review. Earth Sci Rev 142:1–17. https://doi.org/10.1016/J.Earscirev.2014.12.002

Nehlich O, Borić D, Stefanović S, Richards MP (2010) Sulphur isotope evidence for freshwater fish consumption: a case study from the Danube Gorges, SE Europe. J Archaeol Sci 37:1131–1139. https://doi.org/10.1016/j.jas.2009.12.013

Nehlich O, Fuller BT, Jay M, Mora A, Nicholson RA, Smith CI, Richards MP (2011) Application of sulphur isotope ratios to examine weaning patterns and freshwater fish consumption in Roman Oxfordshire, UK. Geochim Cosmochim Acta 75:4963–4977. https://doi.org/10.1016/j.gca.2011.06.009

Ostrom PH, Fry B (1993) Sources and cycling of organic matter within modern and prehistoric food webs. In: Engel MH, Macko SA (eds) Org Geochem. Plenum Press, New York, pp 785–798

Parnell AC, Inger R, Bearhop S, Jackson AL (2010) Source partitioning using stable isotopes: coping with too much variation. PLoS One 5: e9672. https://doi.org/10.1371/journal.pone.0009672

Peterson BJ, Howarth RW, Garritt RH (1985) Multiple stable isotopes used to trace the flow of organic matter in estuarine food webs. Science 227:1361–1363. https://doi.org/10.1126/science.227.4692.1361

R Development Core Team (2007) R: a language and environment for statistical computing. In. R Foundation for Statistical Computing, Vienna, Austria

Raven JA, Scrimgeour CM (1997) The influence of anoxia on plants of saline habitats with special reference to the sulphur cycle. Ann Bot 79:79–86

Rees CE, Jenkins WJ, Monster J (1978) The sulphur isotopic composition of ocean water sulphate. Geochim Cosmochim Acta 42:377–381. https://doi.org/10.1016/0016-7037(78)90268-5

Reese DS (2005) Whale bones and shell purple-dye at Motya (Western Sicily, Italy). Oxf J Archaeol 24:107–114. https://doi.org/10.1111/j.1468-0092.2005.00227.x

Rennenberg H (1984) The fate of excess sulfur in higher plants. Annu Rev Plant Physiol 35:121–153. https://doi.org/10.1146/annurev.pp.35.060184.001005

Reynard LM, Henderson GM, Hedges REM (2010) Calcium isotope ratios in animal and human bone. Geochim Cosmochim Acta 74: 3735–3750. https://doi.org/10.1016/j.gca.2010.04.002

Richards MP (2002) A brief review of the archaeological evidence for Palaeolithic and Neolithic subsistence. Eur J Clin Nutr 56:1262. https://doi.org/10.1038/sj.ejcn.1601646

Richards MP, Fuller BT, Sponheimer M, Robinson T, Ayliffe L (2003) Sulphur isotopes in palaeodietary studies: a review and results from a controlled feeding experiment. Int J Osteoarchaeol 13:37–45. https://doi.org/10.1002/oa.654

Rowe JH (1969) The sunken gardens of the Peruvian coast. Am Antiq 34: 320–325. https://doi.org/10.2307/278415

Scherer HW (2009) Sulfur in soils. J Plant Nutr Soil Sci 172:326–335. https://doi.org/10.1002/jpln.200900037

Simpson IA (1985) Stable carbon isotope analysis of anthropogenic soils and sediments in Orkney. In: Fieller NJR, Gilbertson DD, Ralph NGA (eds) Palaeoenvironmental investigations: research design, methods and data analysis. British Archaeological Reports International Series 258, Oxford, pp 55–65

Sparks J (2009) Ecological ramifications of the direct foliar uptake of nitrogen. Oecologia 159:1–13. https://doi.org/10.1007/s00442-008-1188-6

Szpak P (2014) Complexities of nitrogen isotope biogeochemistry in plant-soil systems: implications for the study of ancient agricultural and animal management practices. Front Plant Sci 5: 288. https://doi.org/10.3389/fpls.2014.00288

Szpak P, Longstaffe FJ, Millaire J-F, White CD (2012a) Stable isotope biogeochemistry of seabird guano fertilization: results from growth chamber studies with maize (*Zea mays*). PLoS One 7:e33741. https://doi.org/10.1371/journal.pone.0033741

Szpak P, Longstaffe FJ, Millaire J-F, White CD (2014) Large variation in nitrogen isotopic composition of a fertilized legume. J Archaeol Sci 45:72–79. https://doi.org/10.1016/j.jas.2014.02.007

Szpak P, Millaire J-F, White CD, Longstaffe FJ (2012b) Influence of seabird guano and camelid dung fertilization on the nitrogen isotopic composition of field-grown maize (*Zea mays*). J Archaeol Sci 39: 3721–3740. https://doi.org/10.1016/j.jas.2012.06.035

Takahashi J, Kalland A, Moeran B, Bestor TC (1989) Japanese whaling culture: continuities and diversities. Marit Anthropol Stud 2:105–133

Tanz N, Schmidt H-L (2010) δ34S-value measurements in food origin assignments and sulfur isotope fractionations in plants and animals. J Agric Food Chem 58:3139–3146. https://doi.org/10.1021/jf903251k

Tcherkez G, Tea I (2013) $^{32}S/^{34}S$ isotope fractionation in plant sulphur metabolism. New Phytol 200:44–53. https://doi.org/10.1111/nph.12314

Trust BA, Fry B (1992) Stable sulphur isotopes in plants: a review. Plant Cell Environ 15:1105–1110. https://doi.org/10.1111/j.1365-3040.1992.tb01661.x

van der Merwe NJ, Williamson RF, Pfeiffer S, Thomas SC, Allegretto KO (2003) The Moatfield ossuary: isotopic dietary analysis of an Iroquoian community, using dental tissue. J Anthropol Archaeol 22: 245–261. https://doi.org/10.1016/S0278-4165(03)00038-2

Vander Zanden MJ, Rasmussen JB (1999) Primary consumer $\delta^{13}C$ and $\delta^{15}N$ and the trophic position of aquatic consumers. Ecology 80: 1395–1404. https://doi.org/10.1890/0012-9658(1999)080[1395:PCCANA]2.0.CO;2

Yi-Balan SA, Amundson R, Buss HL (2014) Decoupling of sulfur and nitrogen cycling due to biotic processes in a tropical rainforest. Geochim Cosmochim Acta 142:411–428. https://doi.org/10.1016/j.gca.2014.05.049

Zazzo A, Monahan FJ, Moloney AP, Green S, Schmidt O (2011) Sulphur isotopes in animal hair track distance to sea. Rapid Commun Mass Spectrom 25:2371–2378. https://doi.org/10.1002/rcm.5131

Zhao FJ, Knights JS, Hu ZY, McGrath SP (2003) Stable sulfur isotope ratio indicates long-term changes in sulfur deposition in the Broadbalk experiment since 1845. J Environ Qual 32:33–39. https://doi.org/10.2134/jeq2003.3300