



Isotopic insights into quinoa agriculture at an Andean hillfort town (cal AD 1250–1450)

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Abstract

Quinoa (*Chenopodium quinoa*) agriculture has been a cornerstone of highland Andean diets for thousands of years, but it has received relatively little attention from archaeologists studying diet through stable isotope analysis. In this study, we present the largest sample published to date ($n = 49$) of archaeological carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope ratios in quinoa, as well as single samples of cactus (*Opuntia* spp.), wild bean (*Trifolium amabile*), and potato (*Solanum tuberosum*) from a Late Intermediate Period (cal AD 1250–1450) hillfort town in the western Lake Titicaca basin. Quinoa $\delta^{15}\text{N}$ averages $+8.83\text{‰} \pm 2.17$, indicating that agricultural fields at this site were fertilized with camelid manure, but values were significantly higher in samples recovered from high-status compounds than low-status ones. This suggests that high-status groups within the community had larger camelid herds and/or older fields that had been improved with fertilizer for longer periods of time, possibly allowing their plants to be more productive than those of lower-status groups. Mean quinoa $\delta^{13}\text{C}$ was $-23.95\text{‰} \pm 0.72$, which indicates that plants were not significantly more water-stressed than modern or historic comparative samples grown with similar methods. This concurs with paleoclimate data suggesting that the environment surrounding this settlement was in a period of drought recovery during the occupation. Alternatively, fertilizer may have allowed plants to combat the effects of drought without recycling sub-stomatal CO_2 . This study is an important contribution to research on foodways in the ancient Andes and non-cereal grain-dependent societies more broadly.

Keywords *Chenopodium quinoa* · Pastoralism · Stable isotopes · Manuring · Palaeoclimate · Andean Altiplano

Introduction

Quinoa (*Chenopodium quinoa* Willd.) is a nutritious and hardy staple crop that has sustained communities of the Peruvian and Bolivian Altiplano through millennia of climatic fluctuation and socio-political change (Miller et al. 2021). Nutritionally, this pseudo-cereal (non-grass seed or grain) ‘superfood’ has high protein content, contains all

eight essential amino acids, is gluten-free, and has a high micronutrient content (Repo–Carrasco et al. 2003). Moreover, because it was domesticated in an arid, high-altitude environment, it is productive in a wide range of climates and regions. Yet, to date, it has received relatively little attention in archaeological literature relative to maize agriculture (Bruno and Whitehead 2003; López and Recalde 2016; Langlie 2020; Miller et al. 2021). Fortunately, its nutritional and ecological benefits have attracted the interest of food scientists, leading to a growing body of research on the physiology of modern quinoa (Stikić et al. 2015; Alandia et al. 2016; Gámez et al. 2019). This study synthesizes plant science of modern quinoa and archaeological chemistry of cereal grains to interpret new isotopic data on ancient quinoa.

The past two decades have seen considerable advancements in archaeological plant chemistry, with an emphasis on the role of animal manure fertilizers, detected using nitrogen stable isotopes, in ancient agricultural systems (Ferrio et al. 2005; Bogaard et al. 2007; Szpak et al. 2012;

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Szpak 2014; Nitsch et al. 2015; Treasure et al. 2016). This is a particularly important line of inquiry in the South American Andes, where manure, particularly seabird guano, has historically been an important source of wealth (Szpak et al. 2012; Gootenberg 2014; Santana–Sagredo et al. 2021). Additionally, archaeologists have begun to leverage stable carbon isotopes in C_3 plants as an indicator of ancient water availability (Wallace et al. 2013; Styring et al. 2016; Nitsch et al. 2017). To date, these efforts have been primarily focused on cereal grains such as wheat and maize (DeNiro and Hastorf 1985; Boyd et al. 2008; Szpak et al. 2012, 2013). Thus, non-cereal staples such as tubers, legumes, and pseudo-cereals represent a prominent gap in the current literature.

Samples analysed in this paper come from a site known as Ayawiri (Fig. 1) and predominantly date to the Andean Late Intermediate Period (LIP; AD 1100–1450). The LIP coincides with the global Medieval Climate Anomaly, during which, according to numerous lines of evidence, precipitation in the Central Andes was lower than average from approximately AD 800 to 1300 (Abbott et al. 1997; Binford et al. 1997; Mélice and Roucou 1998; Thompson et al. 1998, 2013; Calaway 2005; Arnold et al. 2021a; Guédron et al. 2023). Additionally, the LIP in much of the Andean highlands saw political balkanization, endemic warfare (Covey 2008; Arkush and Tung 2013), and a shift away from maize agriculture (Schreiber 1987; Williams 2002; Miller et al. 2021). In place of maize, communities emphasized camelid herding as an economic base, which was better-suited to drier conditions and socio-political decentralisation (Stanish 2007; Kellett 2016; Arnold et al. 2021b).

With this political and economic re-organization, communities relocated from valley bottoms to defensible hillforts called *pukaras* - of which Ayawiri is an example (Fig. 2). This settlement style became common in the latter half of the LIP on the Altiplano of Peru and Bolivia, and seems to have been designed for defensibility against potential invaders and to facilitate pastoral access to high-altitude grazing lands (Arkush 2011, 2020). Subsistence at Ayawiri relied heavily on camelid herding and the cultivation of tubers and chenopods, all of which were able to thrive at these higher elevations (Bruno 2014; Nielsen 2018; Langlie and Capriles 2021; Miller et al. 2021), in rain-fed terraces (Langlie and Arkush 2016; Langlie 2020).

Using carbon and nitrogen stable isotopes from charred plant remains, we address four important gaps in scientific understanding:

1. We characterize the isotopic range of ancient quinoa grains with the largest data set published to date.
2. We test for the presence of manuring and intra-site differences in its practice.

3. We assess the possibility of detecting water stress in ancient quinoa plants through $\delta^{13}C$.
4. We discuss how these new insights should impact the interpretation of dietary isotopes in humans and animals in the ancient Andes.

Background

Nitrogen isotopes

The two stable isotopes of nitrogen ^{15}N and ^{14}N , are distributed differentially in organisms based upon their abundance in the natural environment, the trophic level of consumers, and the metabolic processes of producers. The ratio of the heavier to lighter isotope is measured in parts per mil (‰) relative to a standard (most commonly Ambient Inhalable Reservoir, or AIR) and represented as $\delta^{15}N$. $\delta^{15}N$ is positively correlated with trophic level in animals, with each trophic level typically representing roughly 3–4‰ (DeNiro and Epstein 1981). $\delta^{15}N$ in bone collagen, which reflects the isotopic composition of dietary protein, is commonly used to identify the staple protein sources of archaeological populations (Froehle et al. 2010; Nitsch et al. 2017; Turner et al. 2018). The underlying assumptions of this approach are that terrestrial plant protein has low $\delta^{15}N$ values relative to animal protein, and that the high protein content of animal products has a strong influence over bone collagen values.

Yet $\delta^{15}N$ values in plants vary greatly, both due to the mycorrhizal (fungal) symbionts of different plant taxa (Szpak 2014; Hussain et al. 2018), and the nitrogen content of the soil in which they grow. In particular, nitrogen-rich fertilizers, including animal manures that were used in ancient societies, are high in ^{15}N and can enrich plant values by one trophic level or more (Bogaard et al. 2007; Christensen et al. 2022). Moreover, quinoa has a high protein content relative to other staple crops cultivated in the Andes (e.g. maize, potatoes), at approximately 15% (Abugoch et al. 2008). If fertilized with animal manure, quinoa theoretically presents a similar isotopic profile to terrestrial meat in human bone collagen, confounding simple dietary models based on $\delta^{15}N$. Though no controlled studies to date have specifically evaluated the effects of camelid manure on quinoa plants, plant science studies of quinoa physiology have reported the fertilization protocols used in controlled experiments (Alandia et al. 2016; Rezzouk et al. 2020a). Separately, archaeologists have begun to study the influence of camelid manure on cereal grains (Szpak et al. 2012). Together, we use the research on quinoa fertilization and camelid manure to inform the interpretation of this study.

The impact of manuring on the $\delta^{15}N$ values of crops depends upon the intensity and duration of manuring, as well as the species of animal manure used and the physiological

processes of the crop plant. Szpak et al. (2012) found that six samples of maize grains fertilized with camelid manure were on average 1.8‰ higher, at $+8.1‰ \pm 1.6$, than the control group, which averaged $+6.3‰ \pm 0.3$. Additionally, one meta-analysis of manuring studies found that camelid dung had slightly higher $\delta^{15}\text{N}$ values and greater enrichment in the values of crop plants than did cattle dung, which is a more common choice for modern farmers in the Andes (Alandia et al. 2016; Rezzouk et al. 2020a). Separately, archaeologists have begun to study the influence of camelid manure on cereal grains (Szpak et al. 2012).

Quinoa grown in subsistence farming conditions in the Andean region is likely to provide the most accurate reference data for ancient chenopods. Modern quinoa grains ($n=3$ bulk samples) purchased at markets in northern Peru had an average value of $+7.88‰ \pm 1.35^1$ (Szpak et al. 2013, p 9). It is likely that these crops were fertilized in some capacity, though the type, quantity, and duration is unknown. Modern quinoa grains grown in the Cusco region of Peru ($n=5$) had a value of $+8.44‰ \pm 0.56$ (Turner et al. 2010, p 524). The authors note that these grains were not subjected to chemical fertilizers, but do not comment on the possibility of animal fertilizers. Miller et al. (2021) provide a rare and valuable baseline data set of quinoa, fertilized with camelid manure (the intensity and duration unknown), from the Lake Poopó region of Bolivia, which averaged $+8.1‰ \pm 2.29$ ($n=3$).

Experimental studies provide greater clarity on the influence of fertilizers. In one paper, quinoa plants fertilized with a mixture of cattle and chicken manure had a mean of $+12.53‰ \pm 2.11$ ($n=40$) (Rezzouk et al. 2020b). A study of quinoa plant leaves grown in unmanured conditions in Northwest Argentina were notably lower, ranging from -5.50 to $+6.03‰$ in different varieties, though unfortunately, neither an overall mean nor raw data was provided (González et al. 2011). Additionally, the number of years over which manure is applied to agricultural fields elevates the $\delta^{15}\text{N}$ values of plants (Szpak 2014). Collectively, archaeological and experimental studies suggest that unmanured quinoa plants could be expected to have a mean below $+7.0‰$, while quinoa fertilized with camelid manure, at an intensity and duration significant enough to be detected, could produce a wide range of values above $+7.0‰$. This proposed value may need to be raised in the case of sustained drought, as aridity has a well-documented positive correlation with $\delta^{15}\text{N}$ (Heaton 1987; Aranibar et al. 2004), though, as discussed below, aridity may also be detected

independently through the analysis of stable carbon isotopes (Styring et al. 2016).

Carbon isotopes

Plants absorb the two stable isotopes of carbon, ^{12}C and ^{13}C , in different proportions based on their photosynthetic pathway. The ratio of the heavier to the lighter isotope is typically expressed as $\delta^{13}\text{C}$ and measured in parts per mil relative to a standard (most commonly Vienna Pee Dee Belemnite, abbreviated VPDB). Most plants in the Andes follow the C_3 (Calvin–Benson) pathway, though maize and kiwicha (*Amaranthus caudatus* L.) follow the C_4 (Hatch–Slack) pathway. Plants following the C_3 photosynthetic pathway discriminate strongly against the heavier, less abundant ^{13}C isotope, resulting in $\delta^{13}\text{C}$ values around $-25.5‰$. C_4 plants discriminate less against the heavier isotope, resulting in higher (less negative) $\delta^{13}\text{C}$ values, with an average of $-12.5‰$ (Tykot 2004, p 435). Cacti and succulents follow the CAM photosynthetic pathway, which results in a wide range of $\delta^{13}\text{C}$ values. Under sub-optimal water availability, plant stomata close in order to limit water loss and plants recycle intercellular CO_2 , discriminating less against the ^{13}C isotope and leading to higher (less negative) $\delta^{13}\text{C}$ values (Stroud et al. 2021). The application of nitrogen-rich fertilizers such as animal manure seems to increase average stomatal conductance in crops, including quinoa (Hati et al. 2006; Alandia et al. 2016), thus lowering $\delta^{13}\text{C}$ values (Busari et al. 2016). Interestingly, this suggests that $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ could be negatively correlated in fertilized plants.

The standard measure for carbon discrimination in plants is represented as $\Delta^{13}\text{C}$ (Farquhar et al. 1982), calculated using the atmospheric $\delta^{13}\text{C}_{\text{CO}_2}$ value for the date of the site cal BP (Ferrio et al. 2005). This is similar to a typical $\delta^{13}\text{C}$ measure, but allows for the direct comparison of ancient and modern plant carbon use, and converts the original data to an absolute value (thus, water stress is associated with lower $\Delta^{13}\text{C}$ values, but higher $\delta^{13}\text{C}$ values). For ancient samples, which all dated from 3,800–500 cal BP, we used $\delta^{13}\text{C}_{\text{air}} = -6.4‰$, based on the Monte Carlo average from Antarctic ice cores from this time period (Eggleston et al. 2016). For modern samples, we used the value $\delta^{13}\text{C}_{\text{air}} = -8.0$ to account for the Suess Effect. $\Delta^{13}\text{C}$ values reported were calculated using the following formula:

$$\Delta^{13}\text{C} = \frac{\delta^{13}\text{C}_{\text{air}} - \delta^{13}\text{C}_{\text{plant}}}{1 + \delta^{13}\text{C}_{\text{plant}}/1000}$$

Additionally, there is some evidence that different varieties of quinoa respond differently to drought conditions (Sun et al. 2014; Stikić et al. 2015; Hussain et al. 2018; Gámez et al. 2019). In one study, the leaves of a quinoa variety grown on the hyper-arid coast of Chile discriminated less

¹ Note that, although we report standard deviations, the number of seeds included in each data point varies substantially between studies, and are not consistently reported; i.e. a sample size of $n=1$ could reflect anything from one to a several hundred seeds, so these standard deviations should not be directly compared between publications.

Table 1 Summary statistics of chenopods in each compound (n = 49), uncorrected

Structure	Status	n=	Mean $\delta^{13}\text{C}$	Std. dev $\delta^{13}\text{C}$	Mean $\delta^{15}\text{N}$	Std. dev. $\delta^{15}\text{N}$
6	High	15	-23.74	0.90	9.41	2.29
44	High	7	-24.11	0.56	7.94	1.35
59	High	9	-24.26	0.44	9.86	1.86
72	Mid	7	-24.46	0.58	9.20	1.73
118	Low	8	-23.46	0.26	6.81	1.89
6 formative		3	-23.37	0.25	7.07	3.70
Total		49	-23.95	0.72	8.83	2.17

against ^{13}C under water stress - typically considered a sign of greater water use efficiency - while a variety grown on the Altiplano displayed no overall difference in $\Delta^{13}\text{C}$ under water-stressed conditions as compared to well-watered ones (Gómez et al. 2019). In sum, lower discrimination against ^{13}C should not yet be interpreted as evidence of well-watered conditions based on $\delta^{13}\text{C}$ alone.

Notably, most previous studies of this topic in both plant science and archaeology have focused on irrigated, rather than rain-fed, agriculture (Wallace et al. 2013; Sun et al. 2014; Styring et al. 2016; but see González et al. 2011). This study is based upon the same scientific principles, but $\delta^{13}\text{C}$ is instead used as a proxy for water stress from low precipitation. Due to the currently limited scientific understanding of these complex interactions between environment, plant taxa, and agricultural practices, the results from Ayawiri will be evaluated qualitatively against baseline values from comparable studies and read in concert with climate data.

Published ancient and modern baseline data for chenopod seeds is summarized in Table 2. While there are a number of plant science studies of $\Delta^{13}\text{C}$ in modern quinoa, standard practice is to sample the foliar tissue, which limits this set of comparable data (e.g. González et al. 2011; Gómez et al. 2019). Leaves are only rarely available in ancient plant assemblages, and $\Delta^{13}\text{C}$ in leaves is not directly comparable to values in seeds (Szpak et al. 2013). Szpak and colleagues (2013, p 9) also found that modern quinoa seeds collected in northern Peru had average $\delta^{13}\text{C}$ values of -25.57‰ ($\Delta^{13}\text{C}=18.03\text{‰}$), while Turner et al. (2010) reported that seeds collected in markets in the Cusco region averaged -25.48 ($\Delta^{13}\text{C}=17.94\text{‰}$). Modern quinoa cultivar seeds from the Lake Poopó region of Bolivia averaged -25.60‰ ($\Delta^{13}\text{C}=18.06\text{‰}$), while archaeological chenopods dating from the Formative to Tiwanaku periods in this same region averaged -22.97‰ ($\Delta^{13}\text{C}=17.13\text{‰}$), corrected for charring (Miller et al. 2021). In a foundational study by DeNiro and Hastorf (1985) of plants from the Upper Mantaro Valley of Peru, modern seeds averaged -25.20‰ ($\Delta^{13}\text{C}=17.64\text{‰}$), and ancient chenopods dating from the Initial through Late Horizon periods averaged -24.24‰ ($\Delta^{13}\text{C}=18.45\text{‰}$), when adjusted for charring. The mean $\Delta^{13}\text{C}$ of this comparative dataset (n = 21) is 17.23‰ , so if Ayawiri falls substantially

below this value, it could cautiously be interpreted as evidence of water stress. Conversely, though, high or average $\Delta^{13}\text{C}$ relative to the comparative dataset should not be interpreted as definitive evidence of well-watered conditions until carbon isotope discrimination in quinoa varieties is better understood.

Use of charred plant remains

Charred seeds, like the ones analysed in this study, are the most commonly preserved form of plant remains in high-altitude regions of the Andes (DeNiro and Hastorf 1985). According to methodological research, charred plant remains largely retain the isotopic values that plants possessed in life when charred at the low temperatures ($\sim 200\text{--}300\text{ °C}$) that allow for preservation and taxonomic identification of ancient grains (DeNiro and Hastorf 1985; Charles et al. 2015; Stroud et al. 2023). However, charring slightly enriches measured $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, so whenever it was necessary to directly compare carbonized plant remains to uncarbonized ones, we corrected for the charring effect by subtracting 0.16‰ from $\delta^{13}\text{C}$ and 0.32‰ from $\delta^{15}\text{N}$ (Stroud et al. 2023).

Site overview and environmental context

Ayawiri, also known as Machu Llaqta, is a dense residential complex located at approximately 4,100 m above sea level (m a.s.l.) atop a high, flat mesa in the western Lake Titicaca Basin (Fig. 1). Proyecto Machu Llaqta conducted investigations at Ayawiri between 2009 and 2014. Radiocarbon dates and ceramics place the first occupation in the Upper Formative Period (200 cal BC–cal AD 25) and the second occupation in the Late Intermediate Period (cal AD 1250–1450). Three stone defensive walls protect the fortress from the north, and the site is bounded on all other sides by cliffs. Within the fortress, additional walls separate approximately 100 residential compounds (Arkush 2018). At maximum occupation, it could have housed between one to two thousand residents. Around 1450, the settlement was rapidly abandoned, perhaps as the result of an attack by enemies (Arkush 2017).

Fig. 1 Map of the Lake Titicaca Basin with key locations marked

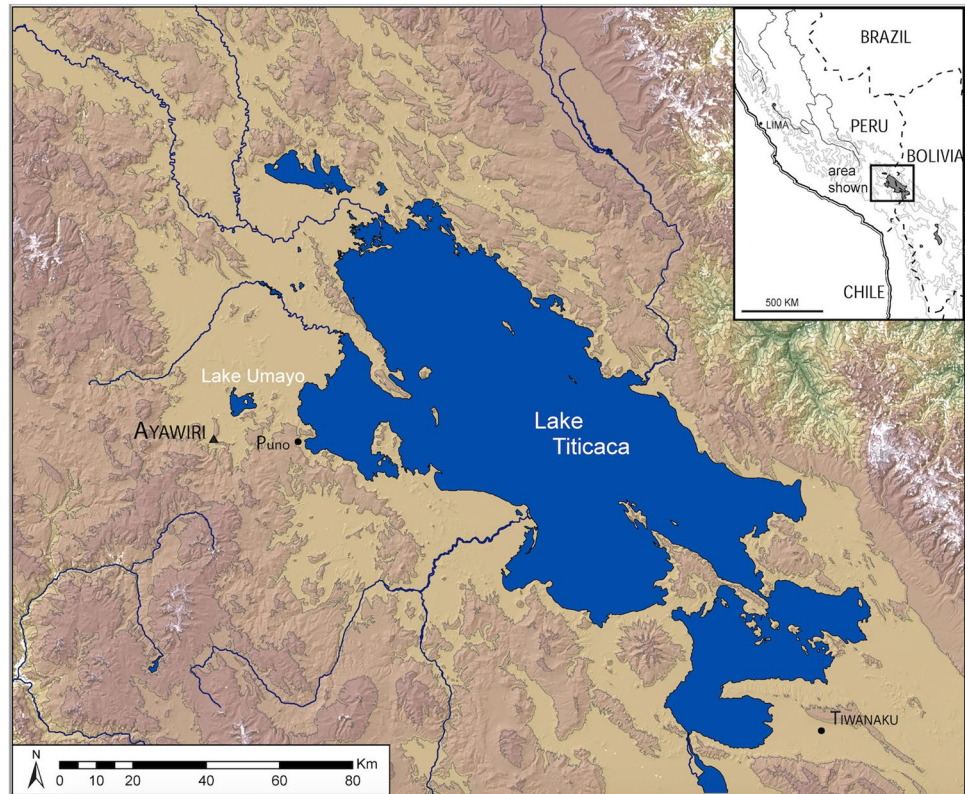


Fig. 2 Photograph of Ayawiri from the southwest

Ayawiri falls within the broader environmental setting of the Andean Altiplano, which stretches from the northern Lake Titicaca Basin in Peru into Central Bolivia, making it the second-largest plateau in the world, with an average altitude above 3,800 m a.s.l. Notably, the site is also situated approximately 8 km from the southern shore of Lake Umayo, a small lake that drains into Lake Titicaca, which could also have provided lacustrine resources, such as reeds and lake fish, for residents of Ayawiri (Baker et al. 2009). Holocene palaeoclimate records from these two nearby lakes allow for a relatively high-resolution reconstruction of the Ayawiri's environmental history. Two recent studies

of sediment cores in the Lago Menor at Lake Titicaca reconstruct lake levels (Bruno et al. 2021; Guédron et al. 2023), and stable oxygen isotopes ($\delta^{18}\text{O}$) from carbonate sediments were previously used to model precipitation at Lake Umayo (Baker et al. 2009). According to these indicators, the Formative Period occupation of Ayawiri saw roughly equivalent - though marginally lower - precipitation in the region of Lake Umayo than the LIP (Baker et al. 2009, p 316), and this is corroborated by a multi-proxy study of lake levels in the Lago Menor of Lake Titicaca (Guédron et al. 2023). Diatom reconstruction of the Lago Menor also suggests somewhat shallow lake levels in the Lago Menor of Lake Titicaca during the second part of this period (Bruno et al. 2021, p 139), but this does not appear to be part of a larger region-wide trend (Arnold 2016; Guédron et al. 2023). According to all three climate reconstructions, precipitation decreased sharply from approximately AD 800–1000 and remained low throughout the early LIP. The period of lowest precipitation varies by study; it is found at approximately AD 1100 according to Baker et al. (2009), around AD 1200 according to Bruno et al. (2021, p 139), and at AD 1000, with an additional dip around AD 1300, according to Guédron et al. (2023). From approximately 1300–1500, according to all three studies, precipitation and lake levels increased steadily. Thus, during the primary time period addressed by this study, it is likely that the region was in a period of recovery from this sustained drought.

Subsistence at Ayawiri

Ayawiri is located in the tundra above 4,000 m a.s.l., known as the *puna*, where camelids (llamas and alpacas) are herded and crops are rarely grown, while its terraces are located in the *suní*, 3,500–4,000 m a.s.l. (though this zone can be extended up to 4,050 m a.s.l. with adaptations such as terracing), where tubers, chenopods, and high-altitude legumes (e.g. tarwi, *Lupinus mutabilis* Sweet) are cultivated (Vidal 2014). These agricultural terraces, built during the LIP, wrapped around three sides of the hillfort (Langlie 2018). Because of its high-altitude, semi-arid environment and nutrient-poor soils, only a narrow range of plants and animals can survive in the Altiplano. Despite this precarity, residents of the Altiplano have developed agricultural systems capable of supporting dense populations for thousands of years. We infer the probable subsistence system at Ayawiri based on previously published archaeological evidence and comparative ethnographic data from modern communities in the south-central highlands.

Based on an abundance of macrobotanical and zooarchaeological remains, diet at Ayawiri seems to have been dominated by C_3 plants (chenopods and tubers) and terrestrial mammals (camelids). Notably, no maize (*Zea mays* L.) was detected at the site (Langlie 2020). Maize agriculture is challenging above 3,500 m a.s.l., requiring large quantities of water and specialized infrastructure to protect against nightly frosts, and evidently the residents of Ayawiri did not prioritize growing or trading for the crop. In contrast, tubers and chenopods are adapted to high altitudes and require less water, and thus were well-adapted to the high, arid Altiplano in the LIP (Vacher 1998; Bruno 2014; Miller et al. 2021). Still, water stress has been associated with lower grain weights in quinoa plants, so the potential impact of drought on chenopod cultivation should not be dismissed (Gámez et al. 2019).

Analysis of camelid dung samples indicates they are rich in companion weeds, demonstrating that llama herds were grazed in agricultural fields, making it likely that their dung was, at least to some extent, deposited on the crops (Langlie and Capriles 2021). This practice is widely documented in modern ethnographic accounts of sectoral fallowing systems, which were common in the Central Andes until at least the end of the 20th century. Such fallowing systems designate most fields as communal pastureland for 2–10 years following the cropping cycle, leaving ample opportunity for natural fertilization (Orlove and Godoy 1986, p 170; Zimmerer 1997). It is likely that these fallow pasturelands were used communally in the ancient past, though both private and communal grazing lands are present in modern comparative contexts (Orlove and Godoy 1986, p 173). Fertilizer, typically animal manure, is added to potatoes, which are the first crop to be planted following a period of fallowing

(Winterhalder et al. 1974, p 95; Orlove and Godoy 1986, p 170; Bruno 2011, p 225). Quinoa is typically planted 1–3 years after potatoes, and does not receive additional fertilizer (Winterhalder et al. 1974, p 95; Bruno 2011). Manure is a particularly valuable resource in this ecological region because it is an efficient means of transforming the biomass of the Altiplano into accessible nutrients for crops (Winterhalder et al. 1974). Manure may be especially crucial for quinoa, which has high nitrogen demands (Zimmerer 1997, p 140).

Perhaps more crucially, manuring adds mass to the soil, thus improving water retention by preventing erosion (Winterhalder et al. 1974; Arriaga and Lowery 2003; Blanco-Canqui et al. 2015). Like many of their modern counterparts, agricultural fields at Ayawiri were rain-fed (unirrigated) terraces, meaning that residents would have managed soil moisture and quality based on their knowledge of seasonality and geology, as well as through practices such as crop rotation and fertilization (Bruno 2011, p 218). Thus, it is likely that the residents of Ayawiri used crops and camelid herds in tandem, allowing fallowed fields to feed their herds and manure from the camelids to nourish their soils, which created a highly efficient system for ensuring sufficient nitrogen and water content for the potato and quinoa crops.

Materials and methods

Sampling

Charred macrobotanical remains were recovered from flotation of soil samples during the 2011 and 2012 seasons of Proyecto Machu Llacta. Excavators collected on average 10 L soil samples from floors, and point samples were collected from smaller, dense contexts (hearths and pits). These soil samples were processed with a SMAP-style flotation device, and original counts and identifications are reported by Langlie (2018, 2020). Samples used in this study ($n = 52$) were selected to reflect a variety of domestic contexts within the site, representing five compounds of high, middle, and low status (Fig. 3, summarized in ESM 1), and depositions in hearths, pits, and floors. The majority ($n = 49$) were quinoa (Fig. 4), as these were by far the most abundant taxon at the site, but potato (*Solanum tuberosum*), cactus (*Opuntia* spp.) seed, and wild bean (*Trifolium amabile*) seeds are represented by one sample each. All samples date to the LIP (cal AD 1250–1450), with the exception of three quinoa seeds from the Upper Formative (200 cal BC–cal AD 25).

Preparation and analysis

At the Cornell Stable Isotope Lab, samples were crushed, homogenised, and weighed into tin capsules. Each sample in

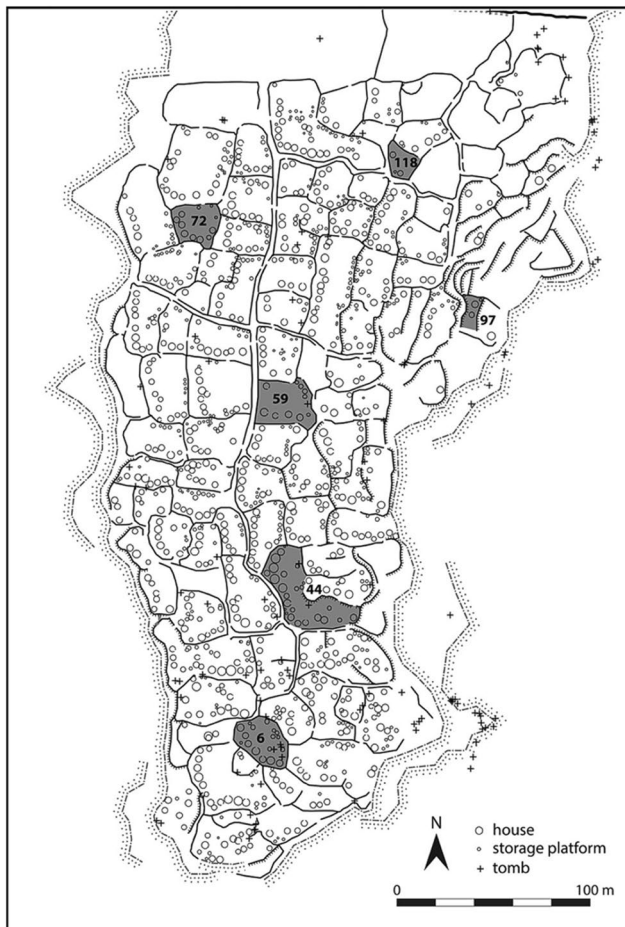


Fig. 3 Map of Ayawiri with patio groups targeted for excavation labelled and highlighted in dark grey

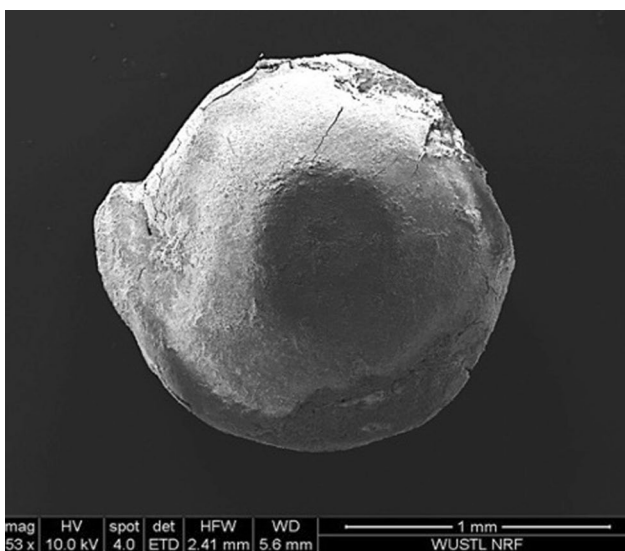


Fig. 4 SEM image of a grain of charred quinoa from Ayawiri

this study represents 2–9 individual seeds, with the exception of a piece of whole potato, in order to ideally reach a weight of 2–3 mg. The samples were washed with water during flotation, then dried and stored in sealed plastic bags or microcentrifuge vials until analysis. Upon visual examination, no soil adhered to individual grains, and while it was not feasible to examine the internal microstructure of each individual quinoa grain, the samples analysed by Langlie (2016, ESM 1) using SEM imagery revealed intact microstructure. We chose to forgo chemical pre-treatment for several reasons. There is currently limited research on appropriate pre-treatments for ancient charred botanical samples, and all documented protocols lead to significant mass loss in the sample, particularly for small seeds like quinoa (Vaiglova et al. 2014; Brinkkemper et al. 2018). In particular, strong acidic treatments can alter the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of the sample, rather than removing contaminants (Vaiglova et al. 2014). Since no known pre-treatment consistently removes the necessary contaminants from carbonized plant remains, and the impact of contaminants, such as carbonates, humic and fulvic acids, and nitrates, has relatively little impact on the resulting isotopic values, we determined that the risks outweighed the benefits (Brinkkemper et al. 2018).

Samples were analysed on a Thermo Delta V isotope ratio mass spectrometer interfaced to a NC2500 elemental analyser. Isotope corrections were conducted using two-point normalization based on in-house standards of KCRN ($\delta^{13}\text{C} = -13.02\text{‰} \pm 0.10$, $\delta^{15}\text{N} = 1.28\text{‰} \pm 0.33$) and CBT ($\delta^{13}\text{C} = -25.58\text{‰} \pm 0.06$, $\delta^{15}\text{N} = 17.48\text{‰} \pm 0.38$). In order to verify that the instrument could accurately measure values with different amplitudes, a chemical methionine standard ($\delta^{13}\text{C} = -26.85\text{‰} \pm 0.27$, $\delta^{15}\text{N} = -0.09\text{‰} \pm 0.69$) was analysed, and found that amplitudes between 200 mV and 15,000 mV have an error associated with a linearity of 0.69‰ for $\delta^{15}\text{N}$ and amplitudes between 200 mV and 10,000 mV for $\delta^{13}\text{C}$ have an error with linearity of 0.27‰. Precision was verified using the in-house standard RICE ($\delta^{13}\text{C} = -29.11\text{‰} \pm 0.10$, $\delta^{15}\text{N} = 0.90\text{‰} \pm 0.18$), run every 10 samples, and was reported to have standard deviations of 0.18‰ for $\delta^{15}\text{N}$ and 0.10‰ for $\delta^{13}\text{C}$.

Ratios are reported as delta-values (δ) unless otherwise noted and measured in parts per mil (‰). $\delta^{13}\text{C}$ was measured relative to VPDB, and $\delta^{15}\text{N}$ was measured relative to AIR. %C, %N, and C:N atomic ratio are reported in ESM 2. Raw isotope data is reported to one decimal place value, while calculations based on these figures are reported to two place values.

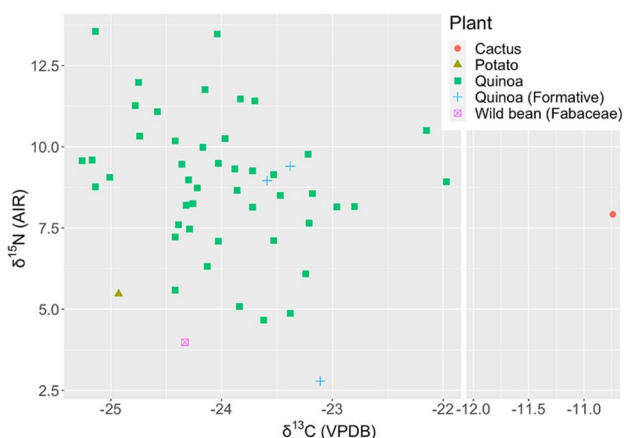


Fig. 5 Scatterplot of all plant results from Ayawiri. Axis breaks were achieved with R code from Xu et al. (2021)

Results

The full data set can be found in ESM 2 and is visualized in Fig. 5. All statistical analyses and data visualization were conducted in R with RStudio version 1.4.1103. The full data set of quinoa samples ($n=49$) had a $\delta^{13}\text{C}$ range of -25.3‰ to -22.0‰ and a mean of $-23.95\text{‰}\pm 0.72$. Quinoa from the LIP ($n=46$) had the same range and a mean of $-23.98\text{‰}\pm 0.73$, and the formative sample ($n=3$) ranged from -23.1‰ to -23.6‰ with a mean of $-23.37\text{‰}\pm 0.25$. In the full quinoa data set, $\delta^{15}\text{N}$ ranged from $+2.8\text{‰}$ to $+13.55\text{‰}$ with a mean of $+8.83\text{‰}\pm 2.17$. For the LIP, values ranged from $+4.7\text{‰}$ to $+13.6\text{‰}$ with a mean of $+8.94\text{‰}\pm 2.04$, and for the formative sample, the range was $+2.8\text{‰}$ to $+9.4\text{‰}$ with a mean of $+7.07\text{‰}\pm 3.70$. The potato sample had a $\delta^{13}\text{C}$ value of -24.9‰ and $\delta^{15}\text{N}$ of $+5.5\text{‰}$, while wild bean was measured at -24.3‰ and $+4.0\text{‰}$, respectively. The cactus seeds, representing the only non- C_3 plant in the sample, a $\delta^{13}\text{C}$ value of -10.7‰ , and $\delta^{15}\text{N}$ of $+7.9\text{‰}$.

Following recommendations from Szpak and Chiou (2020), we tested for a correlation between C:N atomic ratio and $\delta^{15}\text{N}$, and found a weak positive correlation in the full dataset (Spearman's $\rho=0.29$, $p=0.04$) and the chenopod dataset (Spearman's $\rho=0.35$, $p=0.01$). According to Szpak and Chiou (2020), a strong positive correlation in C:N atomic ratio and $\delta^{15}\text{N}$ may be evidence of diagenetic nitrogen loss in the sample, which preferentially retains the heavier ^{15}N isotope. Since this topic requires more research (Szpak and Chiou 2020, p 533), and the correlation is weak to moderate, we do not currently interpret this as evidence of diagenetic alteration. Additionally, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ are negatively, but weakly, correlated in the full dataset (Spearman's $\rho=-0.26$, $p=0.06$) and the chenopod dataset

(Spearman's $\rho=-0.33$, $p=0.02$). The possible implications of this are discussed in section 'Aridity and drought tolerance'.

Table 1 presents $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ summary statistics for each compound. Summary statistics for excavation loci and use contexts are reported in Tables S1-S2 in ESM 1. We used a one-way ANOVA to test for differences in $\delta^{15}\text{N}$ between compounds, and found that at least one pair was significantly different (F statistic = 5.349, $p<0.01$). Tukey-Kramer HSD intervals showed that compound 118 had significantly lower $\delta^{15}\text{N}$ than compounds 59 and 6. Variances were not equal among $\delta^{13}\text{C}$ between compounds, so we tested for difference using a Kruskal–Wallis test, and found that at least one pair was significantly different (chi-squared = 11.691, $p=0.02$). According to a post-hoc Wilcoxon rank sum test with a Bonferroni correction, compound 118 had significantly higher $\delta^{13}\text{C}$ than compounds 59 and 72.

The same analyses were conducted to test for differences between LIP chenopod values in different types of depositional contexts, which were designated as hearths, pits, and floors. No significant differences were detected for $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$.

Discussion

Patterns in manuring of crops

It is highly likely that the chenopods at Ayawiri were fertilized, at least to some extent, with camelid manure, based on other lines of evidence from the site and comparison to previously published data. The mean $\delta^{15}\text{N}$ value observed at Ayawiri falls above our proposed threshold of $+7.0\text{‰}$, and was the second-highest among datasets of quinoa grain grown on small farms in the highland Andes (Table 2) - including the value reported in Miller et al. (2021) for modern quinoa known to have been fertilized with camelid manure. Interestingly, the potato sample had low $\delta^{15}\text{N}$ relative to other plants, at 5.5‰ , running contrary to Bruno's (Bruno 2011, p 225) modern observation that potatoes are the most intensively fertilized crop. This could be an anomaly due to the small sample size of potato, or reflect different priorities in fertilization between past and present farmers.

Higher $\delta^{15}\text{N}$ values are also associated with higher-status (i.e. larger) compounds (Fig. 6). This relationship is statistically significant for high-status compounds 6 and 59 and low-status compound 118. This suggests that some higher-status patio groups practiced more intensive manuring than the broader community, perhaps as a result of possessing larger camelid herds. Alternatively, residents of the higher-status compounds may have planted their crops in older fields that had been improved with manure for a longer period of time, since, in ethnographically documented

Table 2 Grain samples only. Ancient samples corrected -0.16‰ in $\delta^{13}\text{C}$ and -0.32‰ in $\delta^{15}\text{N}$ to account for charring. Modern samples are Suess-corrected $+1.6\text{‰}$ in $\delta^{13}\text{C}$. The formula for $\Delta^{13}\text{C}$ values includes a Suess correction. Comparative data from DeNiro and Hastorf (1985), Turner et al. (2010), Szpak et al. (2013), and Miller et al. (2021)

Publication	Sample age	Mean $\delta^{13}\text{C}$	Mean $\delta^{15}\text{N}$	Mean $\Delta^{13}\text{C}$
(Miller et al. 2021) ($n=3$)	Ancient	-23.13	10.07	17.13
(Miller et al. 2021) ($n=3$)	Modern	-24.00	8.10	18.06
(DeNiro and Hastorf 1985) ($n=5$)	Ancient	-24.40	8.16	18.45
(DeNiro and Hastorf 1985) ($n=2$)	Modern	-23.60	5.35	17.64
(Szpak et al. 2013) ($n=3$)	Modern	-23.97	7.90	18.03
Turner and Armelagos 2010 ($n=5$)	Modern	-23.88	8.44	17.94
Current study LIP ($n=46$)	Ancient	-24.14	8.62	18.18
Current study Formative ($n=3$)	Ancient	-23.53	6.75	17.54

communities, fallow pasture lands included in the sectoral rotation system were accessible to all households in the community, not just those who owned them for cultivation (Orlove and Godoy 1986, p 176; Zimmerer 1997, p 123). Lower mean $\delta^{15}\text{N}$ values in the formative period quinoa sample - prior to the construction of the agricultural terraces - also support this interpretation. These two explanations are not mutually exclusive, and the latter points to the probable importance of “first families” (Arkush 2018, p 16) at Ayawiri. In other words, economic privilege was associated with more nutrient-rich fields, likely resulting in greater crop yields. The economic importance of manure, particularly for agropastoralists, has been observed ethnographically in numerous cultures, including in the ancient and modern Andes (Winterhalder et al. 1974; Jones 2013; Allen 2016; Langlie and Capriles 2021).

Though notable, this status trend is not a strict difference, as compound 44 is a high-status patio group with low mean $\delta^{15}\text{N}$. This suggests that there was variation in manuring practices and/or herd size among higher-status groups, which is consistent with previous findings that heterarchical corporate divisions were the main drivers of social organization at Ayawiri (Arkush 2018; Velasco 2022). In light of the very small sample of low-status compounds, and the exception provided by compound 44, we emphasize that there are other possible explanations for this intra-site variation. While manure is, to some extent, a function of the size of herds possessed by a landholding group, long fallow periods could reduce this impact while also preserving soil quality. It is also possible that some farmers chose not to spread manure evenly across the field. Camelids defecate communally, leading to build-up of manure in centralized areas (Winterhalder et al. 1974, p 102), and since it is likely that camelids at Ayawiri were grazed in fallow agricultural fields (ESM 1 Langlie 2016, p 303), it is possible that some of this original patterning was retained, leading to uneven soil $\delta^{15}\text{N}$ values across the field. Therefore, it is likely that some of the variability visible at Ayawiri is due to agricultural processes without clearly patterned relationships to status or depositional contexts.

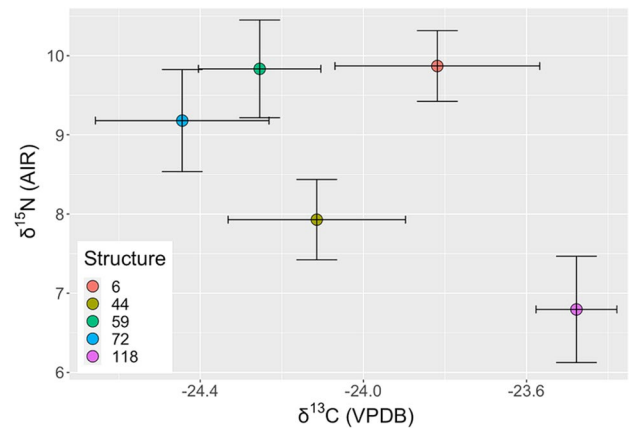


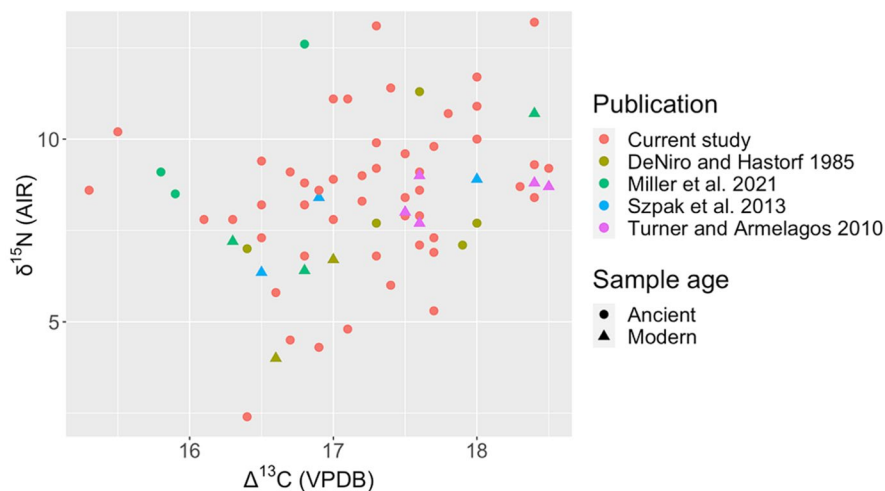
Fig. 6 Means of quinoa plants excavated from each compound. Error bars represent 1 standard error above and below the mean

Aridity and drought tolerance

These results provide robust baseline data for researchers interested in using $\delta^{13}\text{C}$ to infer water stress in quinoa plants. Perhaps most significantly, this study establishes that quinoa grown in traditional, unirrigated highland conditions can be expected to have $\delta^{13}\text{C}$ values of approximately -24‰ ($\Delta^{13}\text{C}=18\text{‰}$, Suess corrected). Carbon isotope discrimination from the present study was similar to (though relatively high among) comparative datasets (Table 2) from a variety of time periods and regions of the Andes.

Note that, though $\delta^{13}\text{C}$ in crop plants has typically been used to infer irrigation practices (Wallace et al. 2013), because the terraces at Ayawiri were rain-fed, the findings presented here address local precipitation. Comparative interpretation suggests that the quinoa cultivated at Ayawiri did *not* employ sub-stomatal CO_2 recycling to a significant degree as a water-saving mechanism, despite the lack of irrigation in the agricultural fields. It is possible that these plants employed other drought-resistant adaptations, including developing deep, dense root systems or employing epidermal cell bladders as water reservoirs (Jensen et al. 2000;

Fig. 7 Carbon isotope discrimination plotted against nitrogen stable isotopes in published data for quinoa grains from DeNiro and Hastorf (1985), Turner et al. (2010), Szpak et al. (2013), and Miller et al. (2021). Ancient samples have been corrected for charring and equations account for the Suess effect



Jacobsen et al. 2009; Sun et al. 2014; Gámez et al. 2019). Or, because the occupation at Ayawiri occurred during a period of drought recovery, the majority of these samples did not experience substantial water stress.

Alternatively, the negative correlation observed between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ runs counter to the expectation that aridity leads to higher $\delta^{13}\text{C}$ and higher $\delta^{15}\text{N}$. As discussed above, manuring increases the stomatal conductance of plants, lowering $\delta^{13}\text{C}$ (Alandia et al. 2016), and improves soil water retention (Arriaga and Lowery 2003; Blanco-Canqui et al. 2015). Szpak and Chiou noted a weak, non-statistically significant negative correlation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in plants from Northern Peru (not including quinoa), which they interpreted as evidence that the high observed $\delta^{15}\text{N}$ values were the result of fertilizer, rather than water stress (2020, p 534). This pattern can also be observed in comparative datasets from the Andes (Fig. 7) and experimental studies of quinoa plants treated with organic fertilizer (Hussain et al. 2018, p 416; Rezzouk et al. 2020b, p 8). Thus, we believe that the significant difference in $\delta^{13}\text{C}$ between compound 118 and compounds 59 and 72 is a result of the differences in manuring levels among these groups.

Use as dietary baseline data

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in bone collagen have most commonly been used to identify the relative consumption of meat and maize in Andean diets (Hastorf 1991; Tung 2021). The findings presented here add to recent cautions against using $\delta^{15}\text{N}$ from human bone *alone* to identify dietary protein source (Szpak et al. 2012; Santana-Sagredo et al. 2021) by demonstrating that manured chenopods, with high protein content and $\delta^{15}\text{N}$ values easily as high as +9‰, could be mistaken for the meat of C_3 -feeding camelids when inferred through human bone collagen. For example, in Miller et al.'s (2021) study of diet on the Altiplano, archaeological chenopods

samples ($n = 3$) had *higher* $\delta^{15}\text{N}$ values ($+10.37\text{‰} \pm 2.21$) than archaeological camelids ($+8.53\text{‰} \pm 1.67$). Researchers should consider the possibility of quinoa as a dietary staple, deferring to additional lines of evidence, such as palaeoethnobotany, zooarchaeology and compound-specific stable isotope analysis before forwarding human bone collagen results as evidence of meat consumption (Szpak et al. 2012; e.g. Miller et al. 2021). In particular, this study calls into question interpretations of meat consumption in cases where animals were foddered or grazed on the same crops that their manure fertilized. In the case of Ayawiri, human bone collagen $\delta^{15}\text{N}$ is more than 4‰ higher than the mean for quinoa, indicating that both manured plants and terrestrial animals likely contributed to dietary protein (Whittemore 2022 and unpubl. data). Enamel apatite $\delta^{13}\text{C}$ confirms that C_4 plants were not significant components of diet (Whittemore et al. 2022).

Conclusions

Our research indicates that the residents of Ayawiri were growing quinoa plants without extreme drought and nourishing their crops with fertilizer to increase yields. Some members of the community, who lived in higher-status compounds, seem to have had access to crops grown on fields steeped in significantly more manure. This may have been a result of larger camelid herds or older agricultural fields in this subset of the community. Still, this does not neatly follow a pronounced status division, in keeping with our understanding of Ayawiri as a society without extreme differences in material wealth.

To our knowledge, this is the largest study to date of the isotopic composition of ancient quinoa remains. Our findings offer a large sample of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for quinoa farmed with pre-colonial methods and emphasize

what many researchers have suspected: that manuring may have a significant impact on $\delta^{15}\text{N}$ in human tissues in the ancient Andes, and should therefore be considered in dietary mixing models. Additionally, these results indicate that, at least in the case of quinoa, a negative correlation between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ may be associated with manuring. Further research on this topic will allow for more accurate interpretations of ancient quinoa production and a better understanding of the numerous ancient societies that relied on C_3 crops.

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Author contributions AFW: Conceptualization, investigation, methodology, visualization, Writing—Original draft; BASL: Funding acquisition, data curation, Writing—Review & editing; EA: funding acquisition, Writing—Review & editing; MCV: Supervision, Writing—Review & editing.

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Data availability All original data used in this article are available in the text and supplementary information.

Declarations

Competing interests The authors have no financial or proprietary interests in any material discussed in this article.

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