Isotope analyses of ancient rice test link between irrigation and complex societies in South Asia

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Abstract

Rice has long been a crucial staple for South Asian human populations and its changing management through time has been closely linked to major changes in social complexity. However, a lack of crop weed preservation has made it difficult to archaeologically distinguish low investment ‘dry’ rice from intensively irrigated paddyfields. Here, we present stable carbon and nitrogen isotope analysis of charred rice grains as a novel proxy for determining water management and manure input. After presenting confirmatory modern baseline data, we apply this methodology to analyse ancient South Asian rice grains, spanning the 3rd millennium BC to the 2nd century BC and the rise of urban landscapes in post-Indus India. We demonstrate the significance of changes in management of this productive staple to social complexity in the region and argue that this approach will greatly advance understandings of linkages between changing human subsistence and social organisation across Asia more widely.

Introduction

Domesticated rice, *Oryza sativa*, is a major staple cereal around the world today, with approximately 8% of global arable land being utilised to cultivate it and to feed more than half of the world's human population (ricecrp.org). Asia alone produces and consumes the vast majority of the crop. Over a billion of the rice-consumers of the continent are in South Asia, where it has held immense nutritional, ecological, and cultural significance since its oldest appearance in the archaeological record in the 7th millennium BC (Tewari et al. 2008). A total consumption of over 142 million tonnes of rice was noted for the region in 2018 (USDA), with 84% of this used directly as food (USDA) contributing to approximately 30% of the total calorie intake per day (FAO computed). Crucially, it is widely considered an affordable food for close to 80% of hungry people in the world (ricecrp.org) and, as such, holds a key political role in South Asia, particularly as climate change increasingly impacts this part of the world (IPCC 2021). Understanding the long-term development of rice farming in the region, and its interaction with cultural preferences, social organisation, and climate dynamics is therefore key for determining potential future outlooks and provision of some of the world's densest populations.

In fact, rice is also one of the most demanding crops (and the most demanding staple), with 35% of the world's irrigation water and 15% of total fertiliser utilised to grow it globally (ricecrp.org). In South Asia alone, it uses millions of hectares of irrigated agricultural area (FAO). Rice is also unique as a crop in that it can be cultivated in a number of different ways which each have their own unique growing conditions and is evident in different agricultural strategies deployed in the region (Figure 1). It can be cultivated both in 'dry' conditions, in dry soils with some groundwater or rainwater, and also in 'wet' growing conditions, where the crop is kept in standing water for most of its growth period (Figure 2). Wet rice varieties require higher investment in the form of adequate irrigation systems, intensive management of wet field systems, and higher labour input (Figure 2). Such high investment is rewarded with the comparatively higher yields of wet rice varieties compared to dry ones (Tao et al. 2016). However, a growing body of research has shown the far-reaching and drastic effects that wet rice agriculture can have, and has had in the past, on accelerating human-induced climate change (e.g., Carlson et al. 2017; Fuller et al. 2011; Kritee et al. 2018; Win et al. 2021).

The water and fertilisation requirements of rice have been argued to have had major impacts on social, political, and cultural organisation in the past as South Asian human societies became increasingly reliant on this productive crop. In particular, transitions to wet rice agriculture have been argued to need higher labour inputs, greater control of water resources in semi-arid and arid environments, and increasing organisation of surplus and harvests. Archaeological evidence has been used to suggest that the spread and adoption of rice in South Asia stimulated social and political complexity, notably in the form of the post-Indus rise of urbanism across the Indian subcontinent (e.g., Morrison 2015; Fuller and Qin 2009). Meanwhile, historical and ethnographic research has shown that control over water resources and rice agriculture became intricately linked to issues of caste, religion, culture, and nationality (Shaw 2005; Shah et al. 2006; Ranjan 2016). The links between rice agro-ecology and processes of social complexity and cultural systems, however, remain poorly understood in South Asia, with either a correlation hypothesised (e.g. Bauer and Morrison 2008; Gililand et al. 2013) or more complex, un-coupled relationships between rice and urbanisation (e.g., Kingwell-Banham 2019).

This is problematic given that South Asia is vast and ecologically diverse, meaning that there is unlikely to have been a simultaneous, sweeping shift from dry to wet rice agriculture. This necessitates robust testing of the linkage between rice growing ecologies and social complexity on a context specific basis. While archaeobotanical and archaeological evidence has been utilised to investigate urbanisation in the region (Kingwell-Banham 2019), issues of the preservation of crop weeds, which have been shown to be most useful in determining past growing ecologies elsewhere, have made direct determination of rice management challenging (Nayak et al., 2021). Stable isotope analysis of ancient crop remains have been used in Southwest Asia and Europe to determine the degree of irrigation and manuring practiced by agricultural communities in the past (e.g. Bogaard et al. 2013; Styring et al. 2017). However, this method has remained under-applied in South Asia (though see Kaushal et al., 2019; Jones et al., 2021) and its effective application to ancient rice grains has yet to be experimentally proven despite the potential significance of this crop to past human social and political organisation. Here, we present stable isotope data from modern rice and archaeobotanical remains from sites in South Asia spanning the 4th millennium BC to the 1st century CE (detailed in Figure 1). In doing so, we show the potential of this methodology to directly test the links between irrigated rice agriculture and increasing urbanisation, not just in the region but also across Asia more widely.

Results

Stable isotope analysis of grains can be used as a proxy for agricultural growing conditions

Grains of local landraces of rice cultivated under distinct growing regimes were collected from different ecological zones in South Asia (see Table 1 in Supplementary Information). A charring experiment (using the procedure outlined by Nitsch et al. 2015) found that there was a standard offset **of x and y** documented between uncharred and charred rice grains for both $\delta^{13}C$ and $\delta^{15}N$ (SI), respectively. As this was constant and predictable **(SD =, SD=),** it provides confidence that the $\delta^{13}C$ and $\delta^{15}N$ of charred archaeobotanical rice grains can be used to as a proxy to reconstruct past growing conditions (as per Nitsch et al. 2015 for barley, lentil, pea, and three varieties of wheat).

The $\delta^{13}C$ values obtained from the experimentally charred rice grains demonstrate clear correlations with watering regimen (Figure 3) **(linear regression)**. These values were converted to $\Delta^{13}C$ using the equation outlined by Farquhar and colleagues (1989). Upon categorising the different landraces according to the growing regime based on modern observations, the $\Delta^{13}C$ values were found to increase with increasing watering. The dry, upland variety had the lowest values ($\Delta^{13}C_{avg}$=18.4‰), while the deepwater varieties had the highest values ($\Delta^{13}C_{avg}$=21.1‰). $\Delta^{13}C$ thus correlated positively with the amount of water the rice crops had during its growth phase (**linear regression)**. An ANOVA test demonstrates clear differences in $\Delta^{13}C$ between different locations whose environments primarily differ in terms of water availability during the growing season (see SI Table 1).

$\delta^{15}N$ values of charred crop remains are commonly correlated with the nitrogen content of the soils the crops were cultivated in, and therefore reflects farming decisions that alter soil nitrogen such as waterlogging or application of manure (Bogaard et al., 2007). Since our modern samples were collected from growing contexts with little to no manure application, we assume that the trends observed in $\delta^{15}N$ values most closely follow the water status of the crop during cultivation. The data we obtained from the modern samples showed the highest $\delta^{15}N$ in the dry, upland rice samples ($\delta^{15}N_{avg}$=5.77‰), and the lowest in the waterlogged, deepwater rice ($\delta^{15}N_{avg}$=3.38‰). An ANOVA test demonstrates clear differences in $\delta^{15}N$ between different locations whose environments primarily differ in terms of watering strategies applied to rice at the different locations (see SI Table 1).

Our modern baseline study demonstrates that the $\delta^{13}C$ and $\delta^{15}N$ of charred rice grains can be used to confidently reconstruct water availability during growth and the watering strategies applied by farmers to rice, respectively. This is significant, as while manuring will complicate interpretations of $\delta^{15}N$ in the past, the combined analysis of $\delta^{13}C$ and $\delta^{15}N$ can be used to pull apart increased manuring and water management by human societies cultivating rice through time. In other words, if $\Delta^{13}C$ increases with increasing water, $\delta^{15}N$ would be expected to decrease in phase climatically. Where $\Delta^{13}C$ increases and $\delta^{15}N$ also increases, it can be assumed that this is a product of increased application of manuring. The baselines provided by these modern values have also been used as a reference for the interpretation of the archaeobotanical data in each region (see Figure 3).

Variability and experimentation in early agriculture: archaeobotanical data 3000 to 1500 BC

The earliest evidence of rice in South Asia is in the form of a husk clot of rice dated to the 7th millennium BC at the Neolithic site of Lahuradewa in north India (Tewari et al. 2008). While this date is undisputed, it is only with the presence of multiple grain and crop processing remains that rice cultivation is considered incontrovertible, and this evidence does not come for several more millennia. The Middle Ganga Plains provides evidence for rice cultivation in several sites from the 3rd millennium BC onwards. The spread of rice outside this region is hypothesised to have occurred only after 1500 BC, facilitated by naturally wet environments that could easily grow the crop with minimal changes (Fuller and Qin 2009; Kingwell-Banham et al. 2018).

The stable carbon isotope data we obtained from the archaeobotanical remains from the early farming sites of Lahuradewa ($\Delta^{13}C_{avg}$=19.84‰, SD =0.59), Senuwar (rice $\Delta^{13}C_{avg}$=19.00‰, SD=1.07; wheat $\Delta^{13}C_{avg}$=18.91‰ SD=0.59; barley $\Delta^{13}C_{avg}$=19.23‰ SD=0.36) and Balu (rice $\Delta^{13}C_{avg}$=18.81‰, SD=1.34; barley $\Delta^{13}C_{avg}$=18.26‰, SD=1.55) located in the Northern Plains of India (designated ‘Plains’ in our study) is lower than the baseline for modern deepwater rice grown in a similar ecological zone ($\Delta^{13}C_{avg}$=21.06‰), and the modern decrue rice from the sub-tropical eastern region of India today (($\Delta^{13}C_{avg}$=20.56‰). The rice isotope data for Senuwar and Balu from the mid 3rd millennium BC (SD=1.07 and 1.34 respectively) suggests significant variation in watering regimes and, likely, an overall reliance on rainwater and flooded alluvial plains for rice cultivation in the Plains.

By 2000 BC, an increase in rice $\Delta^{13}C$ values (=19.84‰ compared to $\Delta^{13}C_{avg}$=18.93‰ for ~2500 BC) and a greater homogeneity of data indicates clear evidence of a move towards irrigation agriculture on the Indus and Ganga Plains, though still below levels indicated by the modern reference data. This is supported by $\delta^{15}N$ data which shows a similar variability in the ~2,500 BC time frame (Lahuradewa $\delta^{15}N_{avg}$=6.26‰ SD=0.99; Senuwar rice $\delta^{15}N_{avg}$=5.09‰ SD=1.91; wheat $\delta^{15}N_{avg}$=2.65‰ SD=1.84; barley $\delta^{15}N_{avg}$=5.51‰ SD=2.19; Balu rice $\delta^{15}N_{avg}$=7.31‰ SD=2.49; barley $\delta^{15}N_{avg}$=5.81‰ SD=0.76). Overall, elevated $\delta^{15}N$ relative to the modern baseline of the region today suggests that manuring was practiced in prehistory. Significantly however, by 2,000 BC, there is a homogenisation and decline in $\delta^{15}N$ relative to the earliest period. In combination with the $\Delta^{13}C$ trends, this $\delta^{15}N$ data thus likely indicates a combination of low input manuring and increased irrigation of rice on the plains at this time.

The early farming sites of eastern India/‘East’ (c. 1300 BC) are similarly highly variable in terms of $\Delta^{13}C$ and $\delta^{15}N$. The $\Delta^{13}C$ data falls below that of the modern baseline of the region and are similar to rainfed rice ($\Delta^{13}C_{avg}$=19.00 ‰ compared to modern irrigated Eastern rice $\Delta^{13}C_{avg}$=20.56‰). Meanwhile, high $\delta^{15}N$ confirms low water input and the use of manure ($\delta^{15}N_{avg}$=7.31‰ compared to $\delta^{15}N_{avg}$=3.52‰ for modern irrigated Eastern rice). Zooarchaeological evidence from the sites (Mohanty et al. 2012) indicates that domesticated bovids and caprids were kept as livestock, and our data additionally shows that their dung was likely incorporated as manure.

Spread of rice facilitated by management of water resources: archaeobotanical data 1500 BC 200 BC

In East India, the $\Delta^{13}C$ and $\delta^{15}N$ results from the Neolithic-Chalcolithic sites of Golbai-Sasan (rice $\Delta^{13}C_{avg}$=19.42 ‰ SD=0.54 $\delta^{15}N_{avg}$=6.12‰ SD= 0.42; horsegram $\Delta^{13}C_{avg}$=17.10‰ SD=0.21 $\delta^{15}N_{avg}$=2.63‰ SD=0.13), Gopalpur (rice $\Delta^{13}C_{avg}$= 18.81‰ SD=0.66 $\delta^{15}N_{avg}$=8.50‰ SD=1.73; horsegram $\Delta^{13}C_{avg}$=17.50‰ SD=0.39 $\delta^{15}N_{avg}$=1.82‰ SD=0.69), and Harirajpur-Bang (rice $\Delta^{13}C_{avg}$=18.83‰ SD=0.68 $\delta^{15}N_{avg}$=7.31‰ SD=1.11; horsegram $\Delta^{13}C_{avg}$=18.62 SD=1.21 $\delta^{15}N_{avg}$=2.17‰ SD=1.17) demonstrate a high degree of variability in cultivation practices (rice $\Delta^{13}C_{avg}$= -25.02‰ SD=0.63; horsegram $\Delta^{13}C_{avg}$= -24.15‰ SD=1.10). Comparison of these data with the modern rice point to a picture of largely opportunistic rainfed agriculture supported by manuring that became less experimental with time (see figure 4). The spread of rice agriculture from the northern Plains to coastal East India is hypothesised to have been facilitated by ecologically similar wet zones that were conducive to the crop (Fuller and Qin 2009). The $\Delta^{13}C$ and $\delta^{15}N$ data from c. 1300-1150 BC East Indian sites and those from the previous period (c. 3000-2000BC) from the Plains are both indicative of a practice of largely rainfed rice agriculture. Additionally, the horsegram data from the sites shows high water status and minimal manuring of the crop. This suggests that while there may have been some experimental supplementation of irrigation, there was likely a continuation of rainfed rice agriculture and manuring associated with livestock grazing in this part of India, i.e., a low input system that innovatively incorporated the different available resources.

In the drier environs of northern and western South Asia, the spread of rice from ~1,200 BC has been associated with social complexity and irrigation infrastructure on the basis of current archaeobotanical data. Our results from the oldest levels (1190–770 BC) at the northern site of Barikot are representative of the key early urbanisation phase of this region. The data obtained from archaeobotanical rice from these levels (rice $\Delta^{13}C_{avg}$=19.65‰ SD=0.80 and $\delta^{15}N_{avg}$=5.01‰ SD=1.99) relative to the local modern dry rice baseline shows that it was irrigated and not grown as a dry, upland crop. This is congruent with the $\Delta^{13}C$ and $\delta^{15}N$ data obtained from lentil samples from the same period which indicates that the abundant water resources of the Swat river valley and the social infrastructure required to manage them were utilised (see Figure and SI Table 1). Moreover, the wheat and legume data from the Neolithic sites of Qasim Bagh and Yunteng from the same region indicate low manuring and high water status, and show an opportunistic agricultural strategy utilising the local resources at hand. Towards the end of this first urbanisation period (c. 640 BC), and before the re-establishment coinciding with the Achaemenid acculturation phase c. 450 BC, wider disruptions to this community are evident in the destruction of the defensive structures (Olivieri et al. 2019). Our results from the archaeobotanical remains reflect this period of social turbulence. The rice $\Delta^{13}C$ results demonstrate a high level of variability

($\Delta^{13}C_{avg}$=18.83‰ SD=1.53), far greater than the previous periods, indicating a reliance on a variety of water management strategies ranging from dry to irrigated cultivation. As social networks and urban infrastructures deteriorated, the previously established agricultural strategies were discarded. Individualised strategies to cultivation appear to have become the common practice, as farmers likely made informed and independent decisions about the use of key resources. As urbanisation is re-established (e.g. in the form of the opulent city of Bazira or Beira in Greek and Latin historical sources, cf. Olivieri et al. 2019), however, centralised urban life made the control and use of agricultural resources feasible once more. The data from the 4th century BC levels, indicates a return to irrigated rice agriculture ($\Delta^{13}C_{avg}$=18.72‰ SD=0.21). Crucially, the prosperity generated by agricultural strategies informed by traditional ecological knowledge and urban centralised resource management may have enabled the city to withstand the 327 BC siege and loot of the city by Alexander and the Macedonian armies.

In the west, by 300-200 BC, the urban sites of Paithan and Ter show $\Delta^{13}C$ values congruent with low levels of irrigation, but not flooded paddy fields like the ones widespread in the region today. Crucially, both sites are located on river banks, and excavated irrigation canals attest to their availability for agriculture around the city. The wheat and barley data from the same time period demonstrate that both crops were cultivated with low water input. In contrast, the $\Delta^{13}C$ values for wheat and barley from older, agropastoral Neolithic sites (c. 2500-1500 BC) from the region point to a high water status for both crops. Additionally, the elevated $\delta^{15}N$ values point to medium amounts of manuring for these earlier sites. The even higher $\delta^{15}N$ values in the urban sites, coupled with the low water status, attests to the use of manure to facilitate greater agricultural output. In the semi-arid environs of the western Indian subcontinent, the earliest farmers deployed all available resources to cultivate crops during the single cropping season. In later time periods, however, as increasing complexity coalesced in the form of large urban centres such as Paithan, the necessity and utility of year round cropping led to a more considered use of agricultural resources.

Additional detail with regards to rice management at these sites is provided by the $\delta^{15}N$ data from the rice grains. The relatively low $\delta^{15}N$ results from both the northern and western sites in our study indicate the relatively high water status for the rice crop cultivated in this more arid region and show a clear increasing trend over a period of two millennia, from 2200 BC to 200 BC. At Barikot, the trend of increasing $\delta^{15}N$ values from 1200 BC to 200 BC coupled with $\Delta^{13}C$ evidence for irrigated rice throughout, points to an increasing input of manure. Similarly, in the western region, when the $\delta^{15}N$ data are compared with the $\Delta^{13}C$ data from the sites, the modern rice datasets, and the archaeological evidence from the sites, it is clear that this steady increase can likewise be attributed to increasing use of manure to increase agricultural output through time. In both of these areas, the environmental conditions are drier than the heartland of early rice agriculture in South Asia, the Middle Ganga Plains. The adoption and inclusion of rice into a Rabi crop focused farming system of wheat and barley, therefore, necessitated a careful management of water resources for a demanding crop. Moreover, adding rice to the existing agricultural milieu would have involved transitioning from a winter/dry-season focused farming to a more intensive, year-round cultivation. Such a transition meant a reduction in fallow periods, and consequently would result in soils that would demand increased input of manure, and, by extension, labour.

Discussion

Stable carbon and nitrogen isotope analysis of bulk archaeological crop samples has been prominently utilised in Southwest Asian and European contexts to explore the growing conditions and agricultural management practices of past societies, from the origins of agriculture to the emergence of urbanism (Bogaard et al. 2013; Styring et al. 2017; Jones et al., 2021). However these methods have not been widely applied beyond these regions and their prominent crops (e.g. wheat, barley, lentils). We have presented the first multi-period, multi-site crop stable isotope data from a variety of ecological zones in South Asia between the 3rd millennium BC and 1st century CE. We also performed a modern study of rice $\delta^{13}C$ (and $\Delta^{13}C$ calculated from $\delta^{13}C$ measurements) and $\delta^{15}N$ from plants growing under different, known cultivation regimes, showing a positive correlation between crop water status and $\Delta^{13}C$ and $\delta^{15}N$, and between manuring and $\delta^{15}N$. We thus demonstrate the utility of an isotopic approach to the detailed exploration of farming practices in South Asia across space and time, and particularly the growing conditions of perhaps the most important crops to be domesticated by human societies in Asia during the Holocene.

The origins and development of rice farming have long been linked to changes in social complexity in South Asia and, specifically, significant focus has been placed on the emergence of wet rice farming as a driver of urbanism and hierarchical social systems in various parts of the Indian subcontinent (e.g., Morrison 2015). Our data, for the first time, enables direct investigation of this hypothesis. We show that some of the earliest examples of rice agriculture in the 3rd millennium BC, including case studies in East India that have often been associated with early experiments with social complexity, were mainly rainfed and involved the application of manure. By contrast, in drier northern regions, it is clear that the first examples of rice agriculture in the drier regions of Northern Pakistan and Western India immediately involved the use of irrigation to cultivate a water-demanding crop. Our results therefore highlight that the multi-isotopic study of rice grains can be used to investigate more nuanced processes of the adoption and spread of rice farming, something necessary given the diverse tempos and trajectories of agriculture known to prevail across the variable climatic and ecological regions of the Indian subcontinent (Fuller et al. 2011; Nayak et al., 2021).

Our methodology and dataset also opens up a series of possibilities in terms of tracking the origins and spread of agriculture across Asia more generally. In China, for example, there remains significant debate in relation to the contexts of the first cultivation of rice, as well as the social and environmental ramifications of paddy field expansion from 3,000-2,000 years ago (Ma et al., 2020). Similarly, in areas such as Cambodia in Southeast Asia, rice has been seen as a critical mainstay of some of the largest pre-industrial urban forms in existence (Castillo et al., 2020). Given that climate change has been argued to have influenced the eventual collapse of centres such as Greater Angkor (Buckley et al. 2010), the application of the methodology presented here to archaeological contexts with rice in these urban landscapes represents an important step forward for determining the ultimate impact on human economies and livelihoods. Our study should stimulate research into the responses of rice growers in a variety of different contexts, potentially providing insights into responses to different climatic and environmental stresses, political organisation, management strategies, and sustainable pathways to cultivation that can help to inform the significant number of humans relying on the significant calories available from this crucial crop in the 21st century.

Methods

Experimental charring of modern grains

Modern rice grains were collected from eight different locations in four different ecological zones (see Figure 1). The samples were collected with the aim of representing the different rice growing ecologies across India today (see Figure 1). Each sample of rice was obtained from a single field under an organic cropping regime and from a single growing season. Each type of rice was subjected to random sub-sampling to obtain a sample of ~50 grains and they were charred using the method described by Fraser et al. (2013). Each of these samples was wrapped in aluminium foil and buried in sand in separate beakers. The beakers were placed in a Thermo Scientific Heratherm Advanced Protocol Security Oven at 245°C for 24 h in the Stable Isotope Laboratory at the Department of Archaeology, Max Planck Institute for the Science of Human History, Germany. Subsequently, the beakers were removed from the oven and allowed to cool down to room temperature. The aluminium packets containing the grains were taken out of the sand and sub-sampled into three separate batches of ten grains each for each type of rice and placed in labels 2 mL Eppendorf tubes. Each of these samples were homogenised for subsequent stable isotope analysis using a Roth Rotilabo® 1.5 mL or 2 mL micropestle.

Pre-treatment of archaeobotanical remains for stable isotope analysis

Rice grains were recovered from the different sites during excavation (See SI Table 1 for full context information). Between two to ten grains per taxon were selected from each period for each site (where possible), and each grain was assigned an individual sample number and placed in a separate, labelled 2mL Eppendorf tube. If the site had only a single period, a single set of grains was taken. Samples were selected in this manner from Balu, Ojiyana, Lahuradewa, Kunal, Senuwar, Ter, Balathal, Harirajpur-Bang, Golbai-Sassan, Gopalpur, Paithan, Qasim Bagh, Yunteng Peng, and Barikot (Bir-kot-ghwandai).

A single grain per set was chosen for assessing contamination (as per Vaiglova et al. 2014). This grain was cleaned of any visible encrustations using a scalpel, placed in a 2mL Eppendorf tube and crushed using a pestle. The homogenised crushed grain was sampled using a spatula for analysis in a Bruker VERTEX 70v FTIR Spectrometer attached with a Bruker Platinum ATR (attenuated total reflection) A225 accessory. The spectra produced for a grain from each site was assessed to identify the presence of carbonate, nitrate, and humic contamination using the method outlined by Vaiglova et al. (2014).

In the samples where contamination was found, they were pre-treated using the recommendations of Vaiglova et al. (2014). In the samples where there was no evidence for contamination of any kind, the remaining grains were cleaned of any visible encrustations using a scalpel. All of the pre-treated samples were crushed in their individual 2mL Eppendorf tubes using a Roth Rotilabo® 1.5 mL or 2 mL micropestle, with the pestle cleaned with methanol between each sample to avoid cross-contamination.

Stable isotope analysis of archaeobotanical and modern samples

The homogenised powders of the charred modern grains and the archaeobotanical remains were weighed into tin capsules for stable carbon and nitrogen isotope analysis in the Stable Isotope Laboratory of the Department of Archaeology, Max Planck Institute for the Science of Human History, Jena, Germany. The samples were analysed in duplicate by EA-IRMS on a ThermoFisher Elemental Analyzer coupled to a ThermoFisher Delta V Advantage Mass Spectrometer via a ConFloIV system. Isotopic values are reported as the ratio of the heavier isotope to the lighter isotope ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) as δ values in parts per mill (‰) relative to international standards, VPDB for $\delta^{13}C$ and atmospheric N_2 (AIR) for $\delta^{15}N$. Results were calibrated against international standards (USGS 40, IAEA N_2, IAEA C_6, and USGS 61). The measured and true values of each of the standards for each run are reported in SI Table 1. Based on replicate analyses long-term analysis of the machine error over a year is ± 0.2‰ for $\delta^{13}C$ and ± 0.2‰ for $\delta^{15}N$. Overall measurement precision was studied through the measurement of repeats of standards (n= 80, ± 0.2‰ for δ13C and ± 0.2‰ for $\delta^{15}N$).

Statistical analyses

Statistical analyses were conducted using RStudio v1.1.456. Full details are presented in SI 2).

References