

Isotope evidence for agricultural extensification reveals how the world's first cities were fed

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This study sheds light on the agricultural economy that underpinned the emergence of the first urban centres in northern Mesopotamia. Using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of crop remains from the sites of Tell Sabi Abyad, Tell Zeidan, Hamoukar, Tell Brak and Tell Leilan (6500–2000 cal BC), we reveal that labour-intensive practices such as manuring/middening and water management formed an integral part of the agricultural strategy from the seventh millennium BC. Increased agricultural production to support growing urban populations was achieved by cultivation of larger areas of land, entailing lower manure/midden inputs per unit area—extensification. Our findings paint a nuanced picture of the role of agricultural production in new forms of political centralization. The shift towards lower-input farming most plausibly developed gradually at a household level, but the increased importance of land-based wealth constituted a key potential source of political power, providing the possibility for greater bureaucratic control and contributing to the wider societal changes that accompanied urbanization.

The emergence of the first urban centres represents a pivotal moment in human history, and much research has focused on changes in the political, social and productive economy that accompanied and likely contributed to this change^{1,2}. In this study we consider the stable carbon isotope ($\delta^{13}\text{C}$) and nitrogen isotope ($\delta^{15}\text{N}$) values of 276 charred cereal grain and 44 pulse seed samples (each comprising 4–25 individual grains/seeds) from the sites of Tell Sabi Abyad, Tell Zeidan, Hamoukar, Tell Brak and Tell Leilan, located in the Khabur and Balikh drainage basins in northern Mesopotamia and dating to between 6500 and 2000 BC (Fig. 1; Table 1). This allows us to investigate how the staple economy supported the new population centres that emerged in the fourth and third millennia BC in northern Mesopotamia, and thus to reconsider wider debates surrounding the agroecology of early urbanism, its sustainability and the role of political centralisation in shaping some of the world's earliest urbanized landscapes.

Strategies to increase crop production

A generalized narrative of agricultural 'intensification' has long held sway in discussion of early urbanization around the world, in part because of emphasis on irrigation-based societies³. Influential research based in southern Mesopotamia^{4,5}, where irrigation is obligatory and associated with high area yields, has encouraged a prevailing view that urban civilization in the rain-fed north was likewise supported by investing higher labour inputs per unit area^{2,6–8}. The productive potential of northern Mesopotamia in recent times, however, has depended on very extensive cultivation, augmented since the First World War by tractors, pump irrigation and agrochemicals, combined with effective systems of mobilization

and transport^{9,10}. When did this process of extensification begin? Was it initiated by the early cities of northern Mesopotamia in the fourth and third millennia BC, or were these early urban centres instead dependent on high-intensity land management like their southern counterparts?

Although not always explicitly defined in discussion of agricultural practice, here we refer to agricultural intensity in terms of labour and resource inputs per unit area of land¹¹, placing the emphasis on the intention to increase outputs (crop yield) rather than outputs *per se*. Agricultural intensification involves an increase in inputs, resulting in increased crop yields per unit area of land. Practices that could have involved high inputs of labour and resources include manuring or middening with human, animal and/or household waste³, controlling weeds through weeding or turning over the soil and/or decreasing the frequency at which land was left fallow¹². Management of the water available to crops, for example through strategic watering or planting of less drought-tolerant crops in better watered settings/soils, may have been another labour- and resource-intensive agricultural strategy in northern Mesopotamia, given its relatively low (about 200–500 mm year⁻¹) and highly variable annual rainfall. Increased agricultural production through agricultural extensification, by contrast, is enabled by significant expansion of the land under cultivation, such that reduction of inputs and yields per unit area are offset by a larger absolute scale of production¹³. Extensification can occur through implementation of labour-saving techniques such as ploughing, specifically through use of specialized plough animals capable of preparing a much larger area for sowing than can be achieved manually by a farming family. Such radical expansion in arable scale requires an additional supply of labour at harvest

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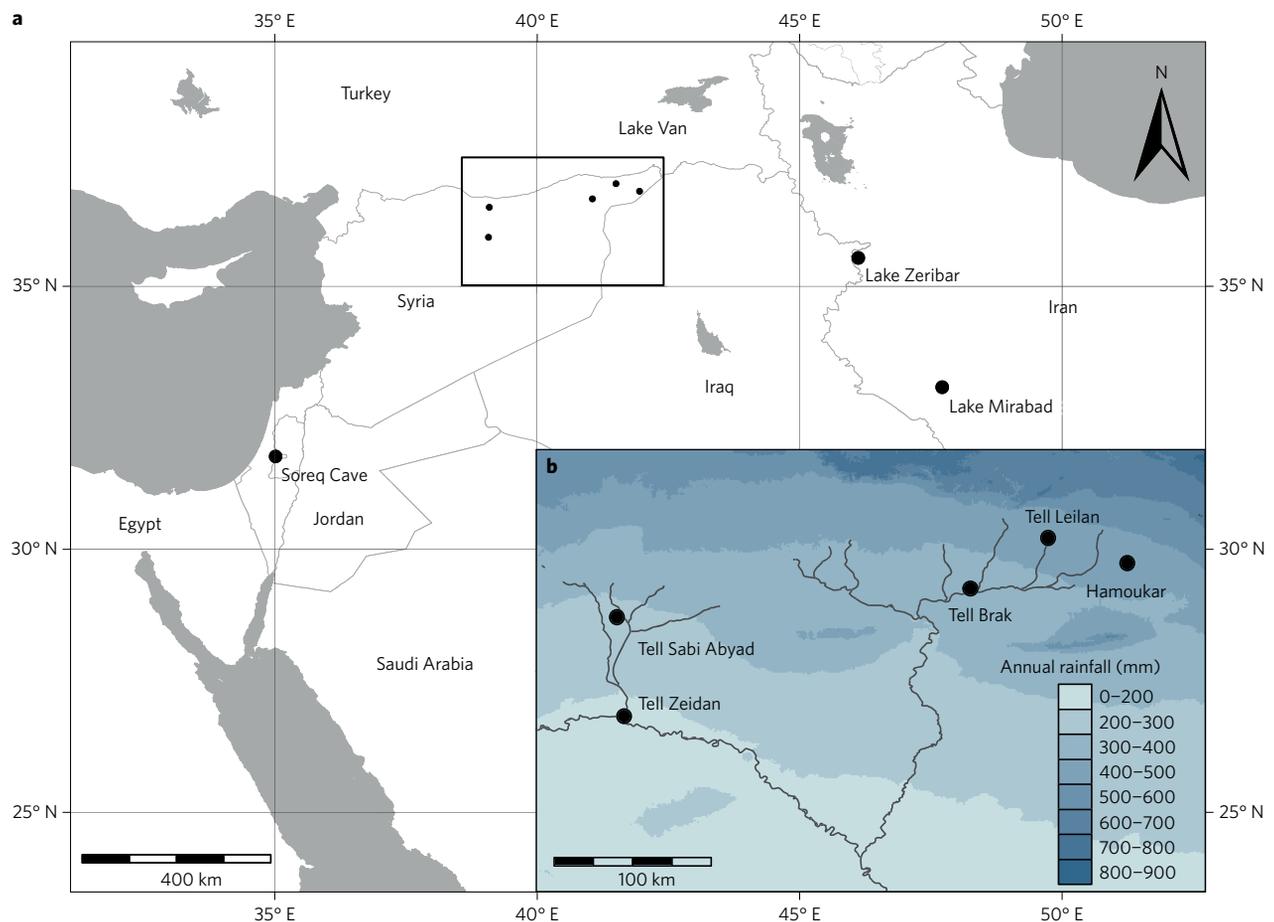


Figure 1 | Geographical location of the study area. a, Overview of northern Mesopotamia. The locations of the palaeoclimate records of Soreq Cave, Lake Van, Lake Zeribar and Lake Mirabad are marked. **b**, The location of the archaeological sites Tell Sabi Abyad, Tell Zeidan, Tell Brak, Hamoukar and Tell Leilan included in this study. Annual rainfall data are derived from interpolation of average monthly climate data for 1960–1990, available from the WorldClim database²⁹.

time, a system that implies a level of organization of labour beyond the immediate household.

Of course, increasing inputs per unit area or expanding the absolute scale of cultivation are not mutually exclusive means of increasing production, and a mixture of the two could be employed to meet the needs of a growing and aggregating population. Indeed, scatters of abraded sherds (proposed to be derived from household waste that was spread on fields to improve their fertility) and ‘hollow ways’ (extensive tracks resulting from confining animal movement to areas between cultivated fields) have been given as evidence for both intensification of manuring and expansion of cultivated land in the third millennium BC^{14,15}, respectively. These sherd scatters and ‘hollow way’ features can be difficult to date, however¹⁶, and it is possible that third millennium practices have obscured earlier evidence of manuring. There is also no way of inferring from this off-site evidence whether cereals, pulses and oil-seed crops were treated differently and thus how their management mapped onto these landscapes.

Directly determining agricultural intensity using crop isotope values. Crop isotope values offer an opportunity to improve our understanding of how agricultural strategies changed under urbanization, delivering a complementary approach to off-site methods that provides direct evidence for the growing conditions of specific crops, better temporal resolution of changing farming practice and more nuanced insight into the relative importance of intensification and extensification among sites and contexts. Crop $\delta^{15}\text{N}$ values largely reflect the $\delta^{15}\text{N}$ value of the soil in which they

are grown, which in turn is strongly influenced by land use history¹⁷. In particular, application of animal manure has been found to increase the $\delta^{15}\text{N}$ values of soil and cereals by as much as 10‰, relating to the intensity—amount and frequency—of manuring^{18,19}, as well as to the type of organic matter—compost, animal manure, household waste—applied²⁰. From now on, we use the term manuring/middening to encapsulate the various means by which organic matter could have been added to the soil. Intensive manuring/middening requires a high input of labour—being heavy to transport and spread—and in modern farming studies usually goes hand-in-hand with other labour-intensive cultivation practices such as weeding and hoeing, since it enhances the tractability (ease of working) of soil²¹. Crop $\delta^{15}\text{N}$ values can therefore act as a proxy for the general intensity of agricultural practice, or labour inputs per unit area.

Crop $\delta^{13}\text{C}$ values reflect the movement of carbon dioxide through the stomata, which in dry climates is most strongly influenced by the water status of a crop during its growth period²². Since rainfall was relatively low at some of the sites and during some of the time periods in our study—and thus marginal for rain-fed farming—it is possible that the water status of crops was manipulated in some way, whether through direct watering or by strategic planting of relatively demanding crops in areas with greater water availability such as the bottoms or slopes of wadis. Crop $\delta^{13}\text{C}$ values can therefore help to elucidate how cultivation was configured in the landscape and identify strategic (and potentially high input) crop management in relation to water resources.

Table 1 | Details of archaeological sites, including location, chronology, settlement size and sample details.

Site	Location (latitude N, longitude E)	Present-day annual rainfall (mm)	Archaeological phase	Date (cal BC)	Settlement size (ha)	Summary of contexts	References
Tell Sabi	39.09, 36.50	280	Early pottery	6700–5850	1	Domestic fills, storage bins	75
Abyad			Neolithic–Halaf				
Tell Zeidan	35.94, 39.08	182	Ubaid–LC2	5300–3850	12	Pyrotechnic features and domestic contexts	54
Tell Brak	36.67, 41.06	363	LC2	4200–3900	55	Mix of workshops, storage, industrial features and monumental buildings	32, 33
			LC3–4	3900–3600	130	Public building, private households and courtyards	32, 33
			LC4–5	3600–3000	45	Large house with southern Late Uruk ceramics	32
			EJ 0	3000–2900	45	Pit cutting LC4–5 house	52
			EJ III–IV	2500–2100	70	Domestic quarters within a ‘high status’ household	
Hamoukar	36.81, 41.96	445	EJ V	2100–2000	45	Potentially ‘public building’	
			LC	3800–3500	15	Area B: tripartite buildings, large ovens	76
Tell Leilan	36.96, 41.51	446	EJ II (Leilan IIIc)	2700–2600	90	Acropolis northwest public stores; Lower Town South residential buildings	53, 55
			EJ III (Leilan IIa)	2600–2300	90	Lower Town South residential buildings	
			EJ IV (Leilan IIb)	2300–2230	90	Lower Town South residential buildings; Acropolis northwest Akkadian palace	
			EJ V (Leilan IIc)	2230–2200	0.1	Acropolis post-Akkadian four-room house	

Present-day annual rainfall is derived from interpolation of average monthly climate data for 1960–1990, available from the WorldClim database²⁹. The date range of each archaeological phase is based on stratigraphic dating and radiocarbon ages. LC, Late Chalcolithic; EJ, Early Jazira.

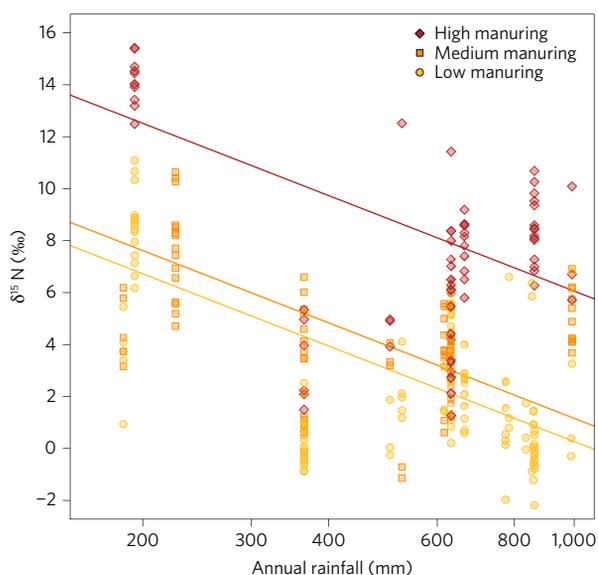


Figure 2 | Modern cereal grain $\delta^{15}\text{N}$ values plotted against the natural log of mean annual rainfall, colour coded by manuring level. The lines represent a fitted linear model relating cereal grain $\delta^{15}\text{N}$ values to mean annual rainfall for each manuring level. Annual rainfall data are derived from interpolation of average monthly climate data for 1960–1990, available from the WorldClim database²⁹.

In this study we aim to provide a better understanding of how agricultural intensity changed during two phases of urbanization in northern Mesopotamia: the Late Chalcolithic period (4400–3000 cal BC) and the Early Bronze Age (2600–2000 cal BC).

Lawrence and Wilkinson²³ have identified three distinct pathways to urbanism, characterized by different site types: ‘hub sites’, which grew slowly in areas of already dense and gradually increasing population (for example, Tell Brak and Late Chalcolithic Hamoukar); ‘endogenous upstarts’, which developed rapidly through the movement of local populations into the urban centre (for example, Tell Leilan); and ‘exogenous upstarts’, which also developed rapidly but in areas with little pre-existing settlement. Within this framework, we can determine whether these contrasting urban trajectories, with different underlying social contexts, entailed different forms of agricultural practice at Tell Brak, Hamoukar and Tell Leilan. The political and productive economies of these sizeable population centres are compared to those of the Late Neolithic settlement of Tell Sabi Abyad (approximately 6500–5200 cal BC) and the Ubaid–Late Chalcolithic 2 town of Tell Zeidan (approximately 5300–3850 cal BC). These data will constrain current models of agricultural intensity and give an unparalleled insight into changing agricultural practice through time, as settlements expanded and contracted, and city-states became established. Moreover, by considering direct evidence of crop growing conditions and farming practice, we hope to provide a counterpoint to top-down ‘elite’ views of agricultural production and move towards a more ‘bottom-up farmer-centric perspective’ of agricultural change²⁴.

Results

Determining manuring intensity at archaeological sites. Aridity can increase plant $\delta^{15}\text{N}$ values²⁵ and it is therefore necessary to take this into account when inferring manuring intensity from cereal grain $\delta^{15}\text{N}$ values. Styring *et al.*¹⁹ used the relationship between modern plant $\delta^{15}\text{N}$ values and rainfall in the eastern Mediterranean²⁶ to adjust expected manuring rates based on $\delta^{15}\text{N}$ values of cereal grains grown on controlled farming plots in temperate Europe^{18,27}. This allows more accurate (and more

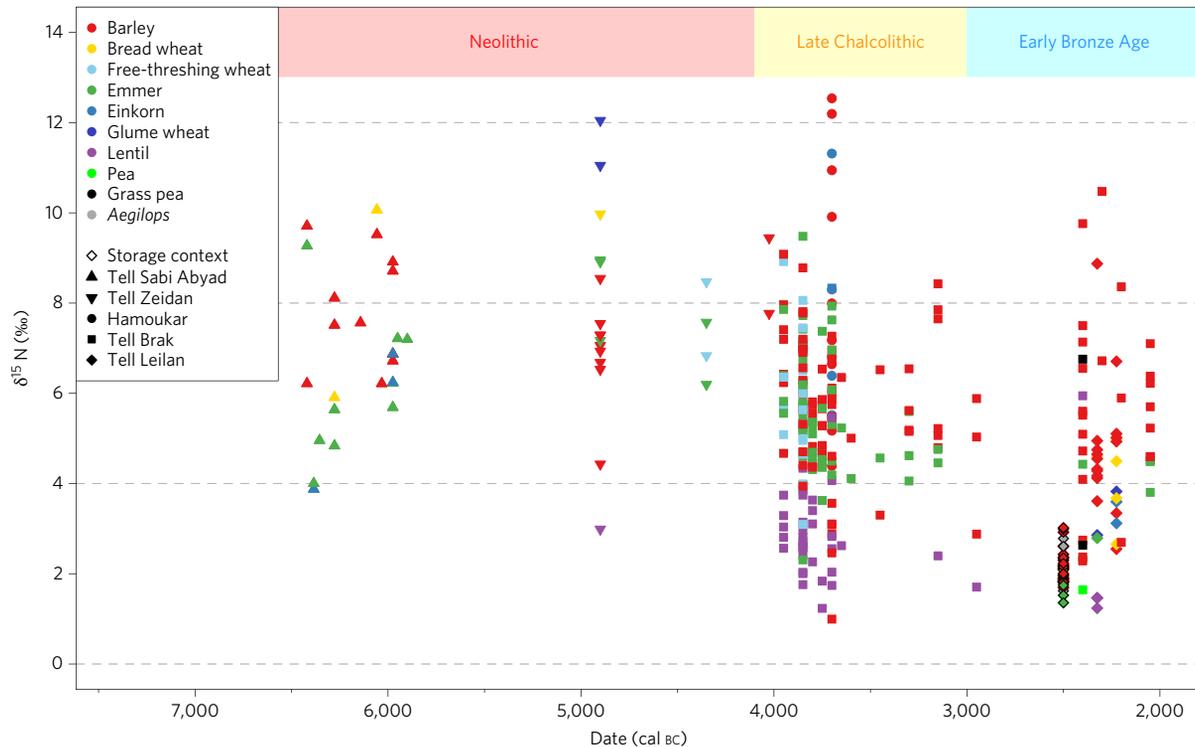


Figure 3 | Archaeological cereal grain sample $\delta^{15}\text{N}$ values plotted against date. The shapes of the symbols vary with site, and the symbols are colour coded by crop taxon. Points outlined in black come from a single storage context. Dating of the crop samples is based on stratigraphic relationships to radiocarbon-dated contexts. More details of the samples are given in Supplementary Table 1.

conservative) estimates of manuring intensity from cereal grain $\delta^{15}\text{N}$ values in semi-arid regions, and here we use the cereal grain isotope data from our studies of modern farming regimens across a wider range of rainfall zones^{18,19,28} to refine this method (Table 2). Figure 2 shows cereal grain $\delta^{15}\text{N}$ values from present-day farming regimens, colour-coded by their known manuring level, plotted against the natural log of annual rainfall derived from interpolation of average monthly climate data for 1960–1990, available from the WorldClim database²⁹. High manuring represents annual manuring of crops at rates equivalent to 30+ tonnes manure ha^{-1} ; medium manuring represents either annual or biennial manuring of crops at lower levels (<20 tonnes ha^{-1}); and low manuring represents no manuring within the last 3+ years. The lines represent a fitted linear model relating cereal grain $\delta^{15}\text{N}$ values to mean annual rainfall for each manuring level.

In this study we use a method called Bayesian multiple imputation³⁰ to assign manuring levels to the archaeological cereal grain samples and simultaneously test for a relationship between these assigned manuring levels and site-related parameters such as size and date (see Supplementary Information for more details). In the first ‘imputation’ stage of multiple imputation, the probability of each cereal grain sample having each manuring level value is derived. Cereal grain $\delta^{15}\text{N}$ values, known manuring levels and rainfall data from our studies of modern farming regimens inform the regression parameters, and these combine with the $\delta^{15}\text{N}$ values and past rainfall ranges of the archaeological material to assign the missing manuring level value (raw archaeological crop $\delta^{15}\text{N}$ values in Fig. 3 and Supplementary Table 1). We also allow for uncertainty in past rainfall level values.

In the second ‘analysis’ stage of multiple imputation we sample multiple assigned manuring level data sets from the manuring level probability distribution we specified at the first stage. We analyse each of these data sets and combine the analyses. In this way our analysis allows for uncertainty in the assigned manuring

levels. At this stage of our analysis, manuring level is an outcome that is potentially explained by site size and other site-specific covariates, such as date. We therefore regress our assigned manuring level data on site size and test for a negative (one sided) effect. Site size is based on field surveys of concentrated surface sherd scatters (as opposed to the continuous and more sparse scatters of small, abraded sherds that have been interpreted as manuring) and targeted excavation (see Table 1 for references). We use a mixed-effects proportional-odds regression model for the three-level ordinal manuring level outcome (low, medium, high). We include a random effect due to the site to model unmeasured site-specific variables that might influence the outcome and to allow for uncertainty in sample date. Given a particular set of assigned manuring levels we carry out Bayesian inference using flat priors, so that all remotely possible parameter values are equally probable *a priori*. The posterior probability distribution for the site size regression parameter is the average of the posterior distributions taken over for each of the assigned manuring level data sets. The final estimated posterior odds (equivalently here, the Bayes Factor) are 5.5 to 1 in favour of a negative effect of site size on the manuring level of cereal grains (Fig. 4). This estimate incorporates all of the important sources of uncertainty and gives substantial support for an effect of site size on cereal grain manuring levels. By contrast, effects due to date are not supported by the imputed data (see Supplementary Information).

The downside of Bayesian multiple imputation is its relative complexity. To make the essential logic of the analysis more accessible, we repeated the analysis using a very simple single imputation approach, and tests based on the maximum likelihood estimator (see Supplementary Information for more details). Based on the fitted linear model relating modern cereal grain $\delta^{15}\text{N}$ values to mean annual rainfall for each manuring level (Fig. 2), each archaeological sample of cereal grains is assigned the manuring level that predicts a $\delta^{15}\text{N}$ value as close as possible to the observed $\delta^{15}\text{N}$ value for that sample. We then regress this

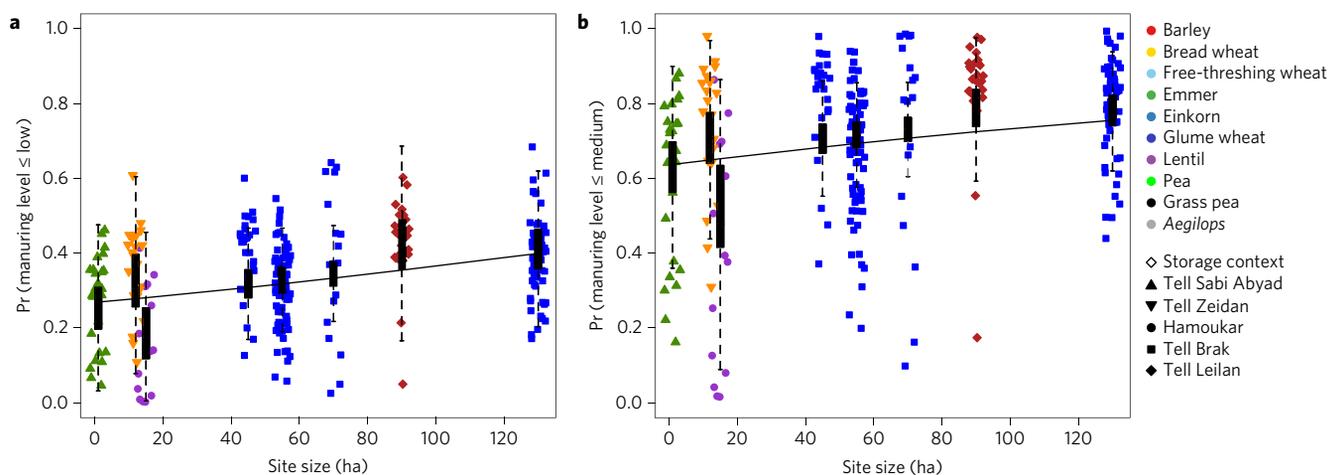


Figure 4 | The probability of an archaeological cereal grain sample having a manuring level m or lower plotted against site size. a, Manuring level $m = \text{low}$. b, Manuring level $m = \text{medium}$. The symbol shape varies with site. The points give the posterior probability for a given cereal grain sample to have a manuring level derived by multiple-imputation that is lower than m . The points are imputed in the first phase of the inference using a normal linear model regressing $\delta^{15}\text{N}$ on the natural log of rainfall and manuring level. Boxes give the quartiles of the fitted posterior probabilities in the proportional-odds regression of manuring level on site size. Dashed whiskers represent $1.5\times$ the interquartile range. The fitted values are offset by site-dependent random effects. Lines show the expected posterior probability that a cereal grain sample at a particular site size has manure level m or lower, and displays the decrease in manuring intensity as site size increases. See Supplementary Information for more detail.

assigned manuring level against site size using the mixed-effects proportional-odds regression model described above and test for a negative effect due to site size (using a Wald test). Again, we find clear evidence for an effect ($p = 0.0034$), but note that this figure does not allow for uncertainty in the imputed manuring levels as the multiple imputation approach does. All our analyses are explained in more detail in the Supplementary Information.

Spatial variation in agricultural strategy. The variability in the $\delta^{15}\text{N}$ values of archaeological cereal grain samples demonstrates that cereals were grown under a range of manuring conditions at each of the archaeological sites (Fig. 3). It seems that we can exclude floodplain cultivation as a potential cause of high $\delta^{15}\text{N}$ values (denitrification during seasonal flooding can result in enrichment of soil ^{15}N (ref. 31)) because $\delta^{15}\text{N}$ values of modern barley grains grown without manure in dry wadi beds that were temporarily flooded following heavy winter rains in 2014 in the south of Morocco are included in both manuring level imputation models. However, isotope analysis of more cereals growing in seasonally flooded settings would be beneficial to test this observation. The large number of cereal grain samples with high $\delta^{15}\text{N}$ values (and thus with a low probability of having a medium or lower manuring level; Fig. 4) at Tell Sabi Abyad demonstrates that manuring/middening formed an integral part of the agricultural strategy from as early as the seventh millennium cal BC in northern Mesopotamia, rather than developing later as a reaction to the need to feed a growing population¹².

Since manure is a heavy and bulky resource to transport, manuring intensity is generally governed by frictions of distance and is thus likely to be highest in plots immediately surrounding settlement areas where animal dung from stabled livestock and/or midden material accumulates⁸. Spatially, then, it is plausible that variable manuring levels within an archaeological site reflect a spectrum of manuring intensity radiating out from the settlement—from intensively managed ‘infield’ areas to more extensively managed fields further away from the urban core. This model mirrors the ‘halos’ of abraded pot sherd scatters surrounding many third millennium BC urban centres and the radiating track ways (‘hollow ways’) that extend beyond these scatters and are believed to delineate the extent of arable cultivation⁸.

There is also evidence that individual households had access to cereals grown under a range of conditions, presumably harvested from plots at varying distances from the site. Barley and glume wheat (einkorn and emmer) grain samples ($n = 8$) representing material stored in separate pots in a single household at LC3–4 Tell Brak, cleaned of weed seeds and sieved for human consumption³², have $\Delta^{13}\text{C}$ values (converted from determined $\delta^{13}\text{C}$ values, see Methods) and $\delta^{15}\text{N}$ values ranging from 15.1–18.4‰ and 1.3–8.6‰, respectively. The large range in crop isotope values demonstrates that household cereal stores derived from plots encompassing a wide spectrum of agricultural intensity. This is consistent with the household having access to land at a range of distances from the urban centre; higher manure inputs could be maintained on plots closer to the settlement and better water retention was likely on soils closer to the Wadi Jaghjagh and Wadi Radd, around 3 km from the site³³. There is also the possibility that some of the crops had come from surrounding villages as a form of tribute³⁴ or that crops were farmed on communal land, but while it cannot necessarily be assumed that this household farmed/owned the disparate plots of land from which their crops came, its access to such variable production points to a risk-buffering strategy in the household’s own interest (for comparison see ref. 35).

Changing agricultural practice. Both the single and multiple imputation approaches show that the probability that cereals received low levels of manure tends to increase as site size increases (Fig. 4). Although attempting to relate settlement size to population is fraught with uncertainty³⁶, given the large range in settlement size considered in this study—from the 1 ha village of Tell Sabi Abyad to the 130 ha sprawl of early fourth millennium BC Tell Brak—we feel it is valid to treat site size as at least a general index of population. The results of the imputation models predict that as settlements in northern Mesopotamia expanded and agricultural production increased, cereals were grown with lower manure inputs per unit area, suggesting that to sustain greater agricultural production, the area of land under cultivation must have increased through a process of extensification. Overall effort expended in manuring plots may well have increased at larger settlements, but the crop isotope results demonstrate that the bulk of the increase in cereal production came from expansion of less

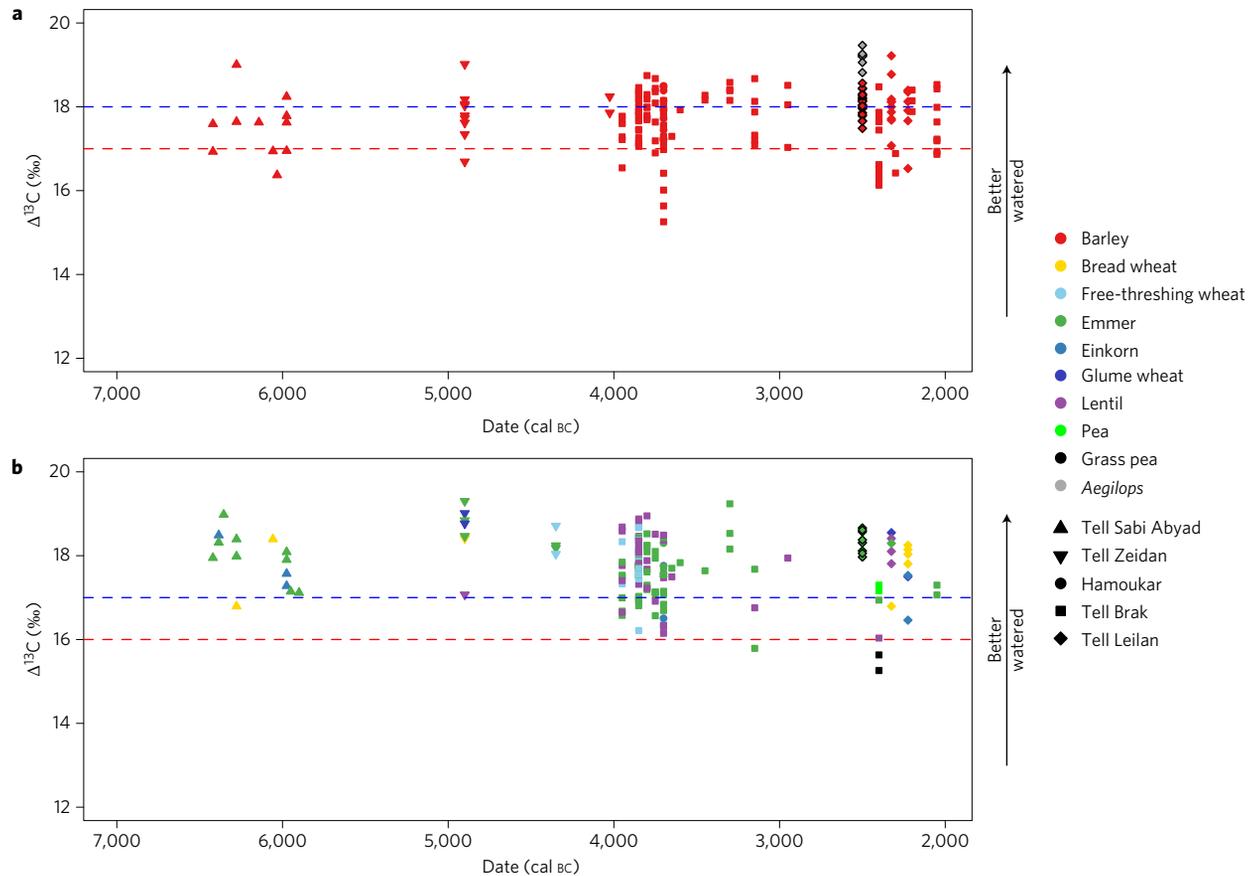


Figure 5 | Archaeological cereal grain and pulse sample $\Delta^{13}\text{C}$ values plotted against date. a, Barley and *Aegilops* grain samples. b, Wheat grain and pulse seed samples. Symbol shape corresponds to the site and symbols are colour coded by crop taxon. Points outlined in black come from a single storage context. Dashed horizontal lines indicate the suggested 'boundaries' between $\Delta^{13}\text{C}$ ranges indicative of crops grown under poorly (low $\Delta^{13}\text{C}$), moderately and well (high $\Delta^{13}\text{C}$)-watered conditions, based on the analysis of present-day crops (for comparison, see ref. 46). *Aegilops* are plotted with barley grains because they were found in a barley store and are therefore assumed to have grown in the same fields as the barley.

intensively manured plots, plausibly those lying beyond the immediate environs of the urban centre.

Our results complement the findings of Araus *et al.*,³⁷ who observed a general trend of decreasing cereal grain $\delta^{15}\text{N}$ values through time at various sites in the Near East. Araus *et al.*³⁷ interpreted this general decrease in cereal $\delta^{15}\text{N}$ values through time as a decrease in soil fertility that could have been caused by myriad potential factors, including agricultural overexploitation, cultivation of marginal lands and reduced manure application. Our model strongly indicates that increasing site size, resulting in a deliberate change in agricultural practice that involved decreased manuring inputs, was a more important factor in decreasing crop $\delta^{15}\text{N}$ values than soil degradation resulting from years of poor agricultural management. This is exemplified by the significant decrease in the manuring level of cereal grains with increasing site size at Tell Brak (see Supplementary Information), despite its larger size in the fourth compared to the third millennium cal BC (Fig. 4a).

Until now, scatters of abraded sherds dated to the third millennium BC have been interpreted as the earliest evidence for manuring at Tell Brak, thus reflecting an intensification of agricultural inputs during the Early Bronze Age^{7,8}. Our new results reveal that the appearance of these sherd scatters does not correlate with an increase in manuring level, at least of cereals. Complementary weed ecological data from Tell Brak show that fertility levels in cereal fields were also relatively low at this time, which is consistent with fields receiving low organic matter/manure inputs³⁸. Thus, sherd scatters are considered to be a visible and persistent sign of the

spreading of organic household waste, but perhaps this practice primarily benefited garden crops^{8,14} and/or was necessitated due to a decrease in the availability of animal manure, perhaps because of competing demands for its use as fuel³⁹. A shift to a highly specialized pastoral economy focused on sheep and goat driven by the commodification of textile production in the third millennium BC⁴⁰, together with the expansion of land under arable production, would have extended herding into more marginal areas, thereby reducing the opportunity for manure collection. The relatively high proportion of cereal grain samples receiving low levels of manure at this time demonstrates that any manuring of fields by animals allowed to graze on stubble or fallow land was not comparable with the increase in cereal grain $\delta^{15}\text{N}$ values that result from deliberate spreading of stall manure.

Crop management in relation to water resources. The archaeological cereal grain and pulse seed $\delta^{13}\text{C}$ values, which have been converted into $\Delta^{13}\text{C}$ values to allow comparison with modern crop studies (see Methods section), can reveal crop management strategies and thus provide insight into how arable land was configured to exploit the hydrology of the landscape. Figure 5 shows the $\Delta^{13}\text{C}$ values of hulled barley, wheat (free-threshing and glume wheats) and pulses (lentil, pea and grass pea) through time. There are no significant changes in crop $\Delta^{13}\text{C}$ values across the time period studied, something that would be expected if the water status of crops was governed solely by the variable rainfall (see palaeoclimate records from for example Lake Van and Soreq Cave^{41,42}).

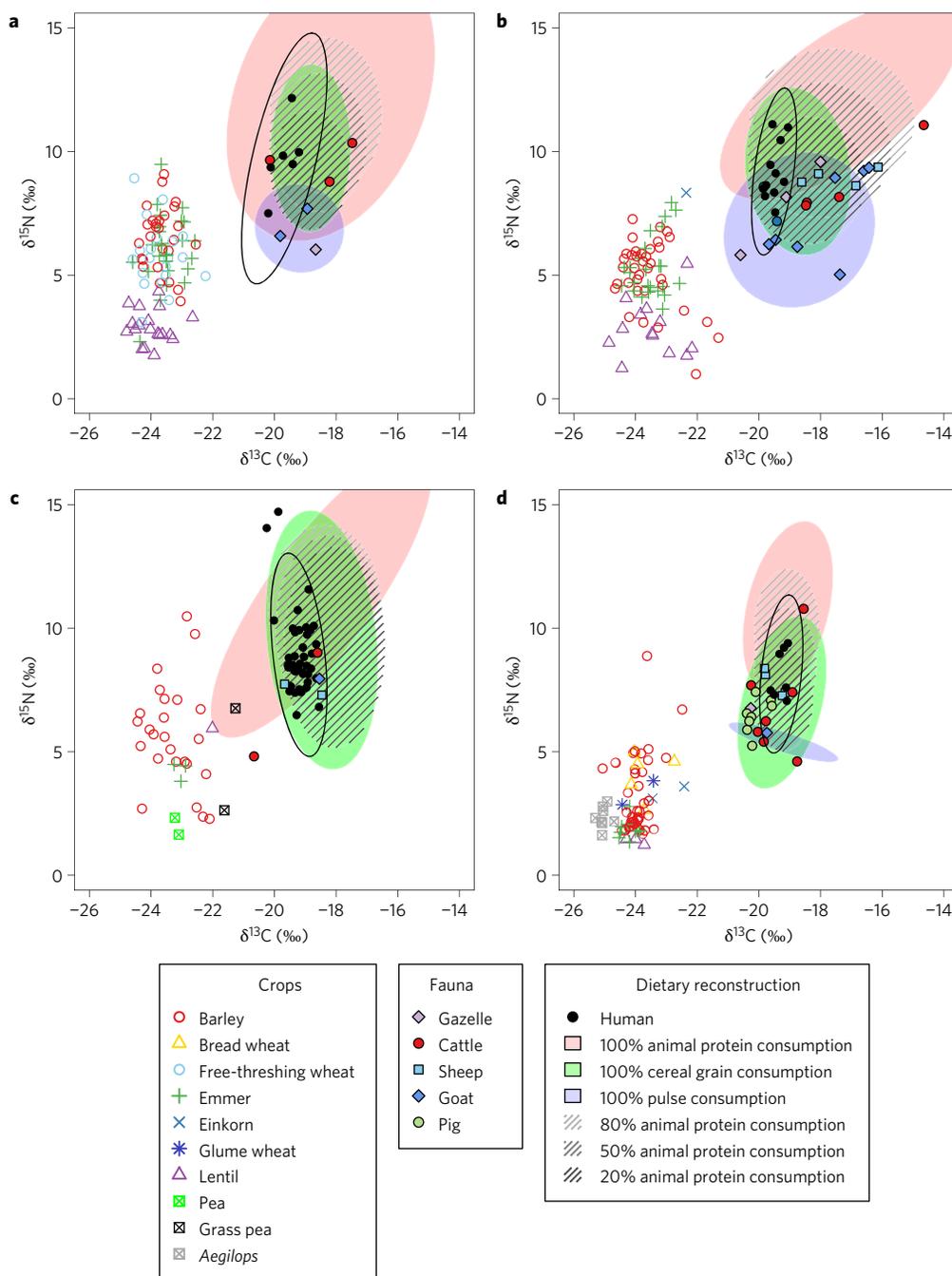


Figure 6 | Human and faunal bone collagen and crop $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values plotted in relation to ellipses representing the expected distributions (mean \pm 2 s.d.) of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of individuals consuming various dietary combinations of cereal grains, pulses and animal products (milk and/or meat). **a**, Late Chalcolithic 2 Tell Brak (about 4200–3900 BC). **b**, Late Chalcolithic 3–4 Tell Brak (about 3900–3300 cal BC). **c**, Early Bronze Age Tell Brak (about 3000–2000 cal BC). **d**, Early Bronze Age Tell Leilan (about 2600–2000 cal BC).

The lack of significant variation in crop $\Delta^{13}\text{C}$ values with time suggests that there was some degree of crop management in relation to water resources at all of the sites. This observation need not necessarily equate to irrigation or deliberate watering, but minimally implies that crops were strategically sown in areas with better water availability, perhaps close to wadis or in soils/areas that retained water, to buffer them from the effects of low rainfall. A study of barley grain $\Delta^{13}\text{C}$ values by Riehl *et al.*⁴³ from archaeological sites across the Fertile Crescent observed lower $\Delta^{13}\text{C}$ values (indicative of poorer water status) during aridification episodes only in the most marginal settings for agriculture. Thus, our results and those reported by Riehl *et al.*⁴³ reflect the difficulty of using crop $\delta^{13}\text{C}$ values as evidence of climate

change *per se*, but instead highlight their potential to measure agronomic adaptation to (independently verified) climate change.

There is also no significant difference in the $\Delta^{13}\text{C}$ values of barley, wheat and pulses (for comparison, see ref. 44). Modern studies have shown, however, that if barley is grown in the same watering conditions as wheat and pulses, it will tend to have a higher $\Delta^{13}\text{C}$ value; offsets range from 1‰ in two-row barley to 2‰ in six-row barley^{45,46}. There are indications that the $\Delta^{13}\text{C}$ values of ancient barley and wheat were also offset, though the magnitude of this offset may have been smaller: six-row barley grain samples ($n=59$) recovered from the archaeological site of Hornstaad-Hörnle IA, Germany (3909 cal BC) have $\Delta^{13}\text{C}$ values

Table 2 | Details of site location, mean annual rainfall and manuring regimens for modern crop samples.

Site	Region	Country	Location (latitude N, longitude E)	Annual rainfall (mm)	Year of collection	Crop species	Manuring regimens	No. of plots
Askov	South Jutland	Denmark	55.53, 9.09	838	2007 and 2008	Two-row hulled barley, bread wheat, emmer, spelt	Low and high	28
Sutton Bonington	Nottinghamshire	UK	52.82, -1.25	632	2007 and 2008	Einkorn, emmer, spelt	Low, medium and high	55
Rothamsted Research	Hertfordshire	UK	51.8, -0.36	655	1852–2004	Two-row hulled barley, bread wheat	Low and high	22
Bad Lauchstädt	Leipzig-Halle	Germany	51.39, 11.83	503	2007 and 2008	Two-row hulled barley, bread wheat	Low, medium and high	11
Sighisoara region	Transylvania	Romania	46.41, 24.92	641	2008	Bread wheat, einkorn	Low and medium	14
Haute Provence	Lubéron/Sault region	France	44.02, 5.42	897	2013	Bread wheat, einkorn	Low	19
Lena district	Asturias	Spain	43.03, -5.76	964	2007	Spelt	Low, medium and high	16
Kastamonu	Kastomonu	Turkey	41.38, 33.70	570	2008	Durum wheat, einkorn, emmer	Low and medium	8
ICARDA	Aleppo	Syria	36.01, 36.93	419	2008	Bread wheat	Low, medium and high	42
Bellota	Ouezzane	Morocco	34.95, -5.54	703	2014	Two-row hulled barley, bread wheat	Low	6
Wadi ibn Hammad	Kerak	Jordan	31.30, 35.63	186	2007	Durum wheat	Low and medium	10
Tighirt	Sidi Ifni	Morocco	29.35, -9.43	272	2014	Two-row hulled barley	Medium	15
Amtoudi (oasis)	Guelmim	Morocco	29.24, -9.19	194	2014	Two-row hulled barley	High	11
Amtoudi (decrue)	Guelmim	Morocco	29.24, -9.19	194	2015	Two-row hulled barley, bread wheat	Low	16

Annual rainfall is derived from interpolation of average monthly climate data for 1960–1990, available from the WorldClim database²⁹.

that are 1.1‰ higher than those of wheat grain samples (naked wheat and einkorn; $n = 120$) grown in the same year⁴⁷. Since these cereals were unlikely to have received additional water inputs, this demonstrates that approximately 1‰ difference in the $\delta^{13}\text{C}$ values of barley and wheat grown in the same watering conditions is observed in these ancient crops. Data from Neolithic Kouphovouno, Greece are also consistent with an offset between two-row hulled barley and wheat, though crop remains here do not derive from a single year's harvest, and were conceivably affected by water management in a Mediterranean zone⁴⁸. When crop $\delta^{13}\text{C}$ values are plotted against watering bands adjusted for the physiological differences between barley (mostly two-row) and other crops (Fig. 5), the majority of the barley grain samples fall into the poor and moderately watered bands defined by Wallace *et al.*,⁴⁶ suggesting that yields were limited by water availability. In contrast, the majority of wheat and pulse samples fall into the well watered band (yields are not limited by water availability). The better water status of wheats and pulses compared to barley further supports an hypothesis of strategic agricultural practice because, at least today, barley generally tolerates drier conditions better than wheat, peas and lentils⁴⁹. This strategy would therefore have maximized overall crop yields in a region where water availability is likely to have presented a key limitation to the optimal growth of crops.

The role of cereals in the economy. Plotting carbon and nitrogen isotope values of human and faunal bone collagen alongside crop isotope values can reveal the importance of crops in the diet of both humans and animals. Figure 6 shows the isotope values of crops, fauna and humans from the Late Chalcolithic and Early Bronze Age at Tell Brak and for the Early Bronze Age at Tell Leilan (faunal and human bone collagen $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values are in Supplementary Table 2). Shaded ellipses represent the expected distributions (mean ± 2 s.d.) of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of individuals consuming various dietary combinations of cereal grains, pulses and animal products (milk and/or meat). The

determined human isotope values (with their mean ± 2 s.d. distribution outlined in black) overlap more closely with the ellipses corresponding to lower animal protein consumption in all periods, suggesting that cereal grains were likely to be an important part of the human diet (for comparison, grain ration records⁵⁰).

During LC3–4, in particular, there is a shift towards higher faunal $\delta^{13}\text{C}$ values, indicating greater C_4 plant consumption (for example, *Cyperus*, *Eragrostis*), which is consistent with grazing on more marginal steppe areas that received lower rainfall. Consumption of C_3 plants growing in areas of lower water availability would also result in higher faunal $\delta^{13}\text{C}$ values, but the particularly high $\delta^{13}\text{C}$ values of some of the fauna ($> -18\text{‰}$), can only be due to consumption of C_4 plants with $\delta^{13}\text{C}$ values of approximately 14‰⁵¹. Tell Brak was 130 ha in extent at this time, and the expansion in cultivation indicated independently by the crop isotope values would plausibly have complemented this movement of animals away from the settlement. In the Early Bronze Age at Tell Leilan, the faunal isotope values overlap entirely with the ellipse for 100% cereal grain consumption, indicating that a significant portion of the herbivore and pig diet could also have been made up of cereal grains. The possibility of cereal grains being grown as fodder for domestic herbivores has been suggested previously on the basis of archaeobotanical findings of large quantities of un-cleaned barley grains at Tell Brak⁵² and Tell Leilan⁵³, and textual references to allocations of cereals as animal fodder⁵⁰.

Discussion

The cereal grain isotope values from archaeological sites in the Khabur and Balikh drainage basins provide a spatial and temporal perspective on changing agricultural practice prior to and during two phases of urbanization. The relatively small settlements of Tell Sabi Abyad and Tell Zeidan (1 and 12 ha, respectively) yielded cereal grains with relatively high $\delta^{15}\text{N}$ values that are consistent with high levels of manuring/middening being practised from as early as the seventh millennium BC, thousands of years

earlier than the appearance of sherd scatters that have previously been the primary evidence for manuring¹⁴. This evidence for early manuring is contrary to evolutionary models of agricultural development that suggest that highly labour-intensive practices such as manuring were only employed when population pressure induced such changes¹². Moreover, we find that strategic crop management in relation to water resources played a key role in cereal and pulse cultivation at even the early sites^{44,54}, likely to be a deliberate means of ensuring adequate production in such a water-limited region. The centrality of cereals in both human and animal diets, as seen in bone collagen isotope values, explains this considerable investment.

Cereal grain nitrogen isotope values reveal that increased agricultural production to support growing urban populations in northern Mesopotamia was achieved by cultivation of larger areas of land, using lower manure/midden inputs per unit area—extensification⁹. This evidence for expansion of arable land is in agreement with off-site survey evidence for extensive arable catchment areas around urban centres (mainly in the third millennium cal BC), indicated by radiating ‘hollow ways’⁸ and regional surveys of site distributions^{10,55}. It also aligns with the economies of scale gained from aggregations of population⁵⁶, since there would have been a supply of labour at crucial bottlenecks in the agricultural year (such as harvest time) that could be mobilized from among other cadres of society (for comparison, Sumerian city-states⁵⁷). Extensification as a means of increasing arable production is in line with the model of extensive agriculture proposed by Weiss^{9,10} for northern Mesopotamia and Halstead¹³ for the provisioning of the urban palatial economies of Late Bronze Age southern Greece, and with evidence for highly intensive management from the initial establishment of farming in Europe²⁷ and the Near East^{19,58}.

The relationship between agricultural intensity and settlement size transcends fourth to third millennium BC differences in social complexity and urban form²³. Thus, the shift towards lower-input farming at larger urban centres most plausibly developed gradually at a household level; as households sought to increase production, plots receiving low labour inputs expanded relative to the more intensively managed plots. Moreover, although our data are consistent with an overall strategy of extensification, this broader framework subsumes a range of behavioural variation that is testimony to a bottom-up as opposed to top-down driver of agricultural change. Individual households seem to have practiced a nuanced and flexible strategy in which (1) the crops planted, (2) where they were planted and (3) labour and material inputs of water and/or manure were all fine-tuned to the specific characteristics of the crop, land and/or soil quality, and the highly variable rainfall circumstances of any given year. This diversity in agricultural practice makes sense as a household risk-buffering strategy but not as an elite-controlled share-cropping regime³⁵.

Nonetheless, this extensive agriculture directly ties production to the amount of land under cultivation—rather than to inputs and therefore to yields per unit area—and heightens the importance of land-based wealth that can be transferred from generation to generation⁵⁹. Thus, extensification could fuel inherited wealth inequality as a potential source of political power. Linking agricultural outputs to land rather than labour inputs also provides a much more tangible measure on which to base levels of taxation/tribute⁶⁰, permitting greater bureaucratic control over surplus, which could have benefited those in political power. Ultimately, this study reveals that the expansive agricultural economy was integral to the development of these first northern Mesopotamian cities, driving—as well as being driven by—the wider societal changes that accompanied this urban trajectory.

Methods

Modern cereal grains. Carbon and nitrogen isotope analysis was carried out on 273 cereal grain samples (each representing a homogenised batch of 50 cereal grains of the same taxon) from 14 farming sites/regions. Details of the site locations,

mean annual rainfall, taxon and manuring regimens for each of the crop samples are in Table 2. The grains in each sample were homogenized prior to isotope analysis using a Spex 2760 FreezerMill.

Archaeological cereal grains. Carbon and nitrogen isotope analysis was carried out on 276 cereal grain and 44 pulse seed samples (each representing a homogenized batch of 4–25 grains/seeds of the same taxon) from five archaeological sites. Twenty-seven of the samples from Tell Brak are the same as those whose $\Delta^{13}\text{C}$ values are reported in Wallace *et al.*⁴⁴; these are identified in Supplementary Table S1. Cereal grains were recovered in a carbonized state from a range of contexts including storage rooms, domestic fires, cooking ovens and floors. The chronology of crop samples was based on stratigraphic dating and radiocarbon ages. Details of the site locations, present-day mean annual rainfall, estimated past rainfall ranges and settlement size can be found in Table 1 and isotopic data for each of the crop samples are listed in Supplementary Table S1.

Human and faunal bone collagen. Carbon and nitrogen isotope analyses were carried out on bone and dentine collagen isolates of 60 humans and 31 herbivores (cattle, gazelle, goat and sheep) from LC2, LC3–4 and EBA Tell Brak and 7 humans, 13 herbivores (cattle, gazelle, goat and sheep) and 8 pigs from EBA Tell Leilan. The occupation periods were selected based on those that had isotope data for crops, fauna and humans. Details of the archaeological contexts in which bones were found, chronology and isotopic data for each of the bone collagen isolates are listed in Supplementary Table 2. Only collagen values with C/N ratios between 2.9 and 3.6 were studied, following quality criteria described by DeNiro⁶¹, and samples with a collagen yield <1% were visually inspected to ensure that they looked like collagen.

Preparation of carbonized crop remains for isotope analysis. Cereal grains and pulse seeds were examined at $\times 7$ –45 magnification for visible surface contaminants, such as adhering sediment or plant roots; these were removed by gentle scraping. Around 10% of the total number of samples from each site were scraped clean, crushed and analysed using Fourier transform infrared spectroscopy with attenuated total reflectance (FTIR-ATR) to look for the presence of carbonate, nitrate and/or humic contamination⁶². Peaks characteristic of carbonate contamination (870 and 720 cm^{-1}) were observed in three of the FTIR spectra of samples from Tell Brak but in none of the FTIR spectra from other sites (Supplementary Fig. 1). It was therefore decided to acid pre-treat all of the crop samples from Tell Brak to dissolve any carbonate⁶³. This procedure consists of treatment with 10 ml of 0.5 M hydrochloric acid at 70 °C for 30–60 minutes, then rinsing in distilled water three times before freeze-drying. This acid pre-treatment does not affect the isotope values of charred grains in the absence of contamination⁶². The samples (both acid pre-treated and untreated) were crushed using an agate mortar and pestle.

Preparation of bones and dentine for isotope analysis. Between 0.5 and 1 g of bone/dentine was cleaned of any visible dirt or carbonate crusts using an aluminium oxide air abrasive. Collagen was isolated using a modified ‘gelatinization method’ based on the methods of Longin⁶⁴ and ultrafiltered prior to isotope analysis.

Stable carbon and nitrogen isotope analysis. All samples (modern and archaeological, crop and bone collagen) were weighed into tin capsules for stable isotope analysis. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of cereal grains and bone collagen were determined on a SerCon EA-GSL mass spectrometer. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of crops were determined in separate runs due to the low %N in the samples. An internal alanine standard was used to calculate raw isotope ratios. For $\delta^{13}\text{C}$ determinations of crops, two-point normalization to the VPDB scale was carried out using four replicates each of IAEA-C6 and IAEA-C7, while for $\delta^{15}\text{N}$ determinations the standards were caffeine and IAEA-N2. For $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ determinations of bone collagen, two-point normalization was carried out using four replicates each of caffeine and seal bone collagen. Reported measurement uncertainties are the calculated combined uncertainty of the raw measurement and reference standards, after Kragten⁶⁵. The average measurement uncertainties for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of crops were 0.09‰ and 0.20‰, respectively. The average measurement uncertainties for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of bone collagen were 0.09‰ and 0.18‰, respectively. These calculations were performed using the statistical programming language R (3.0.2). The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of carbonized crop remains were corrected for the effect of charring by subtracting 0.11‰ and 0.31‰, respectively, from the determined $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, which are the average offsets between uncarbonized crop seeds and those heated for 4, 8 or 24 h at 215, 230, 245 or 260 °C⁶⁶.

Conversion of $\delta^{13}\text{C}$ values to $\Delta^{13}\text{C}$ values. The $\Delta^{13}\text{C}$ values of modern cereal grains were calculated from the determined $\delta^{13}\text{C}$ values ($\delta^{13}\text{C}_{\text{plant}}$) and an average $\delta^{13}\text{C}$ value of atmospheric CO_2 ($\delta^{13}\text{C}_{\text{air}}$) determined from air sampled at weekly intervals during the months that the crops were growing⁶⁷, using the equation below. The $\Delta^{13}\text{C}$ values of archaeological cereal grains were calculated from the determined $\delta^{13}\text{C}$ values ($\delta^{13}\text{C}_{\text{plant}}$) and a $\delta^{13}\text{C}_{\text{air}}$ value approximated by the AIRCO2_LOESS system⁴⁵. The equation was defined by Farquhar *et al.*²²:

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

Estimating past annual rainfall at archaeological sites. We have used the difference between past and present-day annual rainfall at Soreq Cave (location in Fig. 1⁶⁸), estimated from speleothem $\delta^{18}\text{O}$ values and the present-day calibration relationship between speleothem $\delta^{18}\text{O}$ values and rainfall, to adjust present-day annual rainfall at each of the archaeological sites and thus estimate past rainfall at 200 year intervals (a 1‰ decrease in the $\delta^{18}\text{O}$ value of precipitation is equivalent to an increase in annual rainfall of about 200 mm⁶⁸). The uncertainty associated with these estimates is accounted for in the multiple imputation model. Recent work has found that $\delta^{18}\text{O}$ values of rainfall can be affected by the type of precipitation (convective or stratiform) as well as by the amount, and so these estimates of past annual rainfall based on the speleothem $\delta^{18}\text{O}$ values may well be modified in the future⁶⁹. Although estimating past rainfall from a proxy record located around 700 km away from the study sites is problematic, the general trend in climate is similar to that for other lower-resolution proxy records closer to the region. The $\delta^{18}\text{O}$ values of sediments from Lake Mirabad in southwest Iran, Lake Zeribar in western Iran and Lake Van in southeast Turkey (locations in Fig. 1) also show generally wetter conditions than today between about 7000 and 4000 BC followed by a trend towards greater aridity in the third millennium BC^{42,70,71}. Dry phases indicated by higher $\delta^{18}\text{O}$ values about 4500 BC, 3300–3000 BC and 2500–1950 BC are also observed in multiple records⁷².

Visualising potential dietary scenarios. Figure 6 shows the expected distributions (mean \pm 2 s.d.) of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of individuals consuming various dietary combinations of cereal grains, pulses and animal products (milk and/or meat). The expected $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ value distribution of humans consuming 100% cereal grains are estimated by adding consumer diet offsets of 4.8‰⁷³ and 4‰⁷⁴, respectively, to the determined cereal grain $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. The expected $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ value distribution of humans consuming 100% pulses are estimated by adding consumer diet offsets of 4.8‰⁷³ and 4‰⁷⁴, respectively, to the determined pulse $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. Since the consumer diet $\delta^{13}\text{C}$ value offset is smaller between carnivores and herbivores than between herbivores and plants⁷², the expected $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ value distribution of humans consuming 100% animal protein are estimated by adding consumer diet offsets of 0.8‰⁷³ and 4‰⁷⁴, respectively, to the determined faunal bone collagen $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. The expected $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ value distributions of humans consuming mixtures of these diets (20, 50 and 80% animal protein, with the remaining proportion of the diet comprising a 50:50 mix of cereal grains and pulses) are estimated by adding the appropriate consumer diet offsets to the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the dietary components, and multiplying each by their proportion in the diet.

Data availability. Raw $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the archaeological crop samples are given in Supplementary Table 1. Raw $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the modern crop samples used to infer the manuring levels of the archaeological crop samples are given in Supplementary Information (Supplementary Data 2). Full details of the statistical analysis (including R files) are available in Supplementary Section ‘Statistical supplement’, Supplementary Data 1 and 2, and Supplementary Code 1–16. The raw $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the archaeological animal and human bone collagen are given in Supplementary Table 2.

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Author contributions

A.B. conceived the study and contributed to data interpretation and the writing of the manuscript; A.K.S. designed the sampling protocol, carried out analyses, analysed the data and wrote the paper with A.B.; M.C., F.F., M.M.H. and A.Sm. contributed botanical material and data; A.M., G.S. and H.W. contributed data and gave permission for analysis of material; R.M., A.K.P. and J.A.W. contributed faunal material and data; G.K.N. led the statistical analysis and developed the statistical models; M.C.P. and A.So. contributed human bone and dentine material and data. All authors discussed the results and implications and commented on the manuscript at all stages.

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Competing interests

The authors declare no competing financial interests.