

Disentangling the effect of farming practice from aridity on crop stable isotope values: A present-day model from Morocco and its application to early farming sites in the eastern Mediterranean

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Abstract

Agriculture has played a pivotal role in shaping landscapes, soils and vegetation. Developing a better understanding of early farming practices can contribute to wider questions regarding the long-term impact of farming and its nature in comparison with present-day traditional agrosystems. In this study we determine stable carbon and nitrogen isotope values of barley grains from a series of present-day traditionally managed farming plots in Morocco, capturing a range of annual rainfall and farming practices. This allows a framework to be developed to refine current isotopic approaches used to infer manuring intensity and crop water status in (semi-)arid regions. This method has been applied to charred crop remains from two early farming sites in the eastern Mediterranean: Abu Hureyra and 'Ain Ghazal. In this way, our study enhances knowledge of agricultural practice in the past, adding to understanding of how people have shaped and adapted to their environment over thousands of years.

Keywords

agriculture, carbon, crops, manuring, nitrogen, watering

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Introduction

Agriculture has had a major role in shaping landscapes, soils and vegetation, and the beginning of agriculture is one of the candidates for the onset of the Anthropocene (Ruddiman, 2003). Developing a better understanding of early farming practices, revealing the various inputs that were required to make agriculture a success under a range of social and ecological conditions, can therefore contribute to wider questions regarding the trajectory of present-day economies and the agricultural basis of today's subsistence practices.

Although much research has focused on the *when* and *where* of early cultivation and crop domestication (e.g. Price and Bar-Yosef, 2011; Willcox, 2013), less is known about the *nature* of early farming itself, including tillage and weeding, manuring, water management, the longevity of cultivation and the interplay between crop and animal husbandry. Manuring of fields, in particular, implies an investment in the land, since 'topping up' of nutrient levels is only required if plots are long-lived; consecutive crop harvests eventually strip the soil of nutrients, which must be replaced if subsequent crop yields are to be maintained (e.g. Rothamsted Research, 2006: 9). Manuring also implies a certain labour input and a close interdependence between crop and animal husbandry, collection and subsequent spreading of manure requires management of domestic animals and is costly in terms of time and labour (Halstead, 2014). Moreover, since manure is a finite resource, strategic decisions about its application were likely to have been necessary in order to maximise crop yields most effectively, and to enable surpluses to buffer against potential future crop failures (Halstead, 1989).

In the so-called Fertile Crescent region of the eastern Mediterranean, where the earliest crop cultivation and domestication are currently documented (Willcox, 2013), rainfed cultivation is possible but water is the major limitation to growth, as in other semi-arid regions (mean annual rainfall 300–600 mm; Food and Agriculture Organization (FAO), 1987; Noy-Meir, 1973; Webb et al., 1978). Water management practices, whether through location of cultivated plots close to natural water sources or on soils with better natural water retention, or through small-scale hand-watering or larger-scale irrigation works, help to ensure favourable and reliable crop yields (Abbo et al., 2010; Halstead and O'Shea, 1989). Manuring has also been found to improve the water use efficiency of some crop species (Hati et al., 2006). As with manuring, water management practices require strategic planning and management of the cultivated environment, since wetter areas in arid regions are likely to be scarce, and high labour inputs may be required for hand-watering and manipulation of water supplies.

By assessing the likelihood of manuring and water management at early farming sites, it is therefore possible to infer the degree to which early farming societies were investing in the long-term productivity of the land and in the day-to-day maintenance of soil quality, and making strategic decisions about the use of valuable resources such as labour, manure and water. A range of models have been proposed for early farming regimes (summarized in Bogaard, 2005), each with varying degrees of labour input and investment in the land: shifting cultivation, extensive and cultivation, floodplain farming and intensive gardening. Our aim here is not to test these models per se, since this would require incorporation of ecological analysis of arable weed flora alongside stable isotope analysis (cf. Bogaard et al., 2015). Rather, the most relevant contrasts for this discussion of crop isotope chemistry and farming in arid regions are those between relatively low- and high-input forms of cultivation, where management intensity relates to maintenance of crop water and nutrient supply: maintenance of adequate water and soil nutrients would point to relatively intensive management, as in a 'gardening' regime. Naturally favourable water supply and soil fertility would arguably be available in floodplains (Sherratt, 1980), but this model for

early cultivation was developed without ethnographic support (Isaakidou, 2011) and relies on spring sowing for which there is no archaeobotanical evidence (Hillman, 2000; Jones et al., 2013).

In this paper, stable isotope values were determined for barley grains sampled from a series of modern farming plots in Morocco, one of the few (semi)-arid areas of the Mediterranean where traditional agrosystems offer a range of watering and manuring regimes under different annual rainfall conditions, supplementing stable isotope data from modern bread wheat grains grown under a range of controlled irrigation and manuring regimes at an experimental station near Aleppo, Syria (see Fraser et al., 2011; Wallace et al., 2013). These data allow a framework to be developed that will refine current stable isotopic approaches used to infer manuring intensity and crop water status in (semi)-arid regions. This refined method is then applied to charred (carbonized) crop remains (cereal grains and pulse seeds) from two early farming sites in the eastern Mediterranean: Abu Hureyra in the northern Levant, and 'Ain Ghazal in the southern Levant (Figure 5). These sites date to the Pre-Pottery Neolithic and beginning of the Pottery Neolithic (PPNB and PN; c.8800-6000 cal. BC), a period during which both cultivation and herding were becoming established and widespread. The intention is to identify crop isotope signatures that deviate from those expected because of environmental factors (for which rainfall is used as an index), and thus to identify, or discount, potential management practices. In this way, our study aims to enhance knowledge of early agricultural practice, informing wider debates regarding societal changes and subsistence strategies at the advent of farming.

Crop nitrogen isotope values

Crop nitrogen isotope ($\delta^{15}\text{N}$) values integrate a range of environmental and physiological processes, but largely reflect the $\delta^{15}\text{N}$ value of the soil in which they are grown (e.g. Evans, 2001; Högberg, 1997). Studies of modern crops have found that manuring can increase the $\delta^{15}\text{N}$ values of cereals by as much as 10‰, and this increase is related to the intensity – amount and frequency – of manuring (e.g. Bol et al., 2005; Fraser et al., 2011), as well as the type of manure (Szpak, 2014). Based on these modern studies, cereal grain $\delta^{15}\text{N}$ values for high, medium and low/no manuring levels have been estimated (see Figure 3; cf. Bogaard et al., 2013: figure 1). Since changes in manuring level take a number of years to register in crop $\delta^{15}\text{N}$ values (Fraser et al., 2011), contrasts in crop $\delta^{15}\text{N}$ values reflect sustained differences in cultivation, indicating variable investment in the land over many years.

The potential of $\delta^{15}\text{N}$ values of carbonized crop remains from archaeological sites as a means of assessing manuring intensity has been demonstrated in a number of recent studies within temperate Europe (Bogaard et al., 2013; Fraser et al., 2013; Kanstrup et al., 2014; Styring et al., in press; Vaiglova et al., 2014), revealing that farmers in Europe and the Mediterranean practised varying levels of manuring on sites from as early as 5900 cal. BC. Another recent study, by Araus et al. (2014), determined the $\delta^{15}\text{N}$ of pre-domestic and domestic cereal grains from a series of sites in the Near East dating from c. 11,070 cal. BC to the present-day. This study found that cereal grain $\delta^{15}\text{N}$ values decreased over the millennia, a trend that the authors interpret as a reflection of decreasing soil fertility (Araus et al., 2014). A large proportion (45%) of the determined cereal grain $\delta^{15}\text{N}$ values were $> 6\text{‰}$, which in temperate climates would be consistent with intensive manuring (Bogaard et al., 2013). However, 70% of cereal grains with $\delta^{15}\text{N}$ values $> 6\text{‰}$ were associated with pre-domestication cultivation, which although not precluding the possibility of early middening/manuring, is unlikely to have been closely integrated with animal husbandry (cf. Araus et al., 2014). It is possible that the high cereal grain $\delta^{15}\text{N}$ values determined by Araus et al. (2014) are at

least partly due to environmental factors such as aridity, which increases plant $\delta^{15}\text{N}$ values (e.g. Aranibar et al., 2004; Handley et al., 1999; Heaton, 1987). It is therefore necessary to disentangle the effect of manuring from that of aridity before farming practice can be assessed reliably at the archaeological sites in the semi-arid regions where early farming began.

To distinguish the contribution of manure from environmental factors (such as aridity), the approach of recent studies has been to estimate the $\delta^{15}\text{N}$ values of plants consumed by herbivores found at the same archaeological site/phase, by subtracting the offset (c. 4‰; Steele and Daniel, 1978) between consumer and diet from the $\delta^{15}\text{N}$ values of preserved herbivore bone collagen, and to use this as a proxy for an unmanured crop $\delta^{15}\text{N}$ value (Bogaard et al., 2013; Fraser et al., 2013; Styring et al., in press; Vaiglova et al., 2014). This estimated $\delta^{15}\text{N}$ value could differ from the true $\delta^{15}\text{N}$ value of unmanured cereal grains because herbivores consume a range of plant parts, making it difficult to apply a consistent $\delta^{15}\text{N}$ offset (Fraser et al., 2011; Rothamsted Research, unpublished data, 1991). There is also a possibility that domestic herbivores may have received supplementary fodder in the form of crop residues, which may have been manured. Notwithstanding these limitations, the estimated herbivore diet gives an approximate indication of the $\delta^{15}\text{N}$ values to expect for unmanured crops, allowing some assessment of whether any environment-related enrichment of crop ^{15}N is likely, independent of that resulting from agricultural management. In cases where faunal bones are not found or bone collagen is not well-preserved, or the range in herbivore $\delta^{15}\text{N}$ values is so large that an unmanaged plant $\delta^{15}\text{N}$ baseline is not meaningful (e.g. Pearson, 2013), an independent means of inferring a plant isotopic baseline for a particular environment is necessary.

Aridity and nitrogen isotope values

Various studies of soils and vegetation have found a positive correlation between aridity (low rainfall and high evapotranspiration) and $\delta^{15}\text{N}$ values (e.g. Aranibar et al., 2004; Austin and Vitousek, 1998; Craine et al., 2009; Handley et al., 1999; Hartman and Danin, 2010; Heaton, 1987; Swap et al., 2004). The mechanisms of this enrichment remain speculative, but current hypotheses suggest that $\delta^{15}\text{N}$ values relate to the ‘openness’ of the nitrogen (N) cycle: the extent to which N is in excess to plant demand and can therefore be lost through volatilization (c.f. Austin and Vitousek, 1998). Any process that decreases the flux of N into organic matter or increases the flux of N from organic matter to mineral pools favours this volatilization and therefore N loss (Handley et al., 1999). Both plant growth and microbial activity decrease with decreasing soil moisture, producing an excess of readily volatilized mineral-N (Barber, 1995; Stark and Firestone, 1995) whose loss increases the $\delta^{15}\text{N}$ value of the remaining soil-N. This enrichment in soil ^{15}N can be enhanced by progressive cycles of wetting and drying, which also stimulate loss of N in the form of N_2O and NO (Austin et al., 2004). Rainfall thus tends to account for the majority of the variation in $\delta^{15}\text{N}$ values of unmanaged plants (e.g. Craine et al., 2009; Handley et al., 1999; Hartman and Danin, 2010; Swap et al., 2004), but the trend is complicated by differences in mycorrhizal interactions (Craine et al., 2009), soil texture (Aranibar et al., 2004) and plant type (Gebauer and Ehleringer, 2000; Hartman and Danin, 2010).

Crop carbon isotope values

Crop carbon isotope ($\delta^{13}\text{C}$) values reflect the water status of the crop during its growth period (e.g. Araus et al., 1997; Farquhar and Richards, 1984). In C_3 plants, differences in plant $\delta^{13}\text{C}$ values relate to the degree of stomatal conductance, i.e. the rate of passage of CO_2 through stomata, which is regulated according to water availability (Ehleringer et al., 1993; Farquhar et al., 1989).

In well-watered conditions, open stomata freely permit the assimilation of CO₂, allowing discrimination against the heavier ¹³C isotope and resulting in more negative plant δ¹³C values. In drier/poorly watered conditions, closed stomata restrict the assimilation of CO₂, reducing discrimination against ¹³C and resulting in less negative plant δ¹³C values.

The degree of discrimination against ¹³C varies between species (e.g. Ferrio et al., 2005; Flohr et al., 2011; Wallace et al., 2013), so that e.g. barley and wheat grown in the same watering conditions will have different δ¹³C values. Water status itself can be influenced by many factors, including water inputs (i.e. rainfall, anthropogenic watering) and water losses (i.e. evapotranspiration), as well as by the water-holding capacity of the soil. For this reason, Wallace et al. (2013) proposed three broad levels of water status based on crop δ¹³C values: poorly watered crops for which water availability imposes major limitations on growth; well-watered crops for which water is not a major limitation on growth; and moderately watered crops which fall between the two.

When using crop δ¹³C values to infer water status of archaeological crop remains, it is also necessary to account for the change in the δ¹³C value of atmospheric carbon dioxide (δ¹³C_{air}) over time. Today, the δ¹³C_{air} value is c. −8‰ (White and Vaughn, 2011) but it was higher in the past. The δ¹³C_{air} values for the time periods covered by the archaeobotanical samples were therefore approximated by the AIRCO2_LOESS system (Ferrio et al., 2005) and these are given in Wallace et al. (2015). This allows the watering bands established from modern crop watering experiments to be applied to crop remains from different time periods. Crop δ¹³C values are converted to Δ¹³C values by the following equation (Farquhar et al., 1989)

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

Crop isotope values in semi-arid regions: A pilot study in Morocco

This study determines the δ¹⁵N and δ¹³C values of hulled six-row barley grains (*Hordeum vulgare* subsp. *vulgare*) growing in traditionally managed manured and unmanured fields ($n = 34$) receiving different quantities of annual rainfall (190 to 700 mm/yr) and supplementary water from irrigation in Morocco from October, 2013 to March/May, 2014 (Figure 1 and Supplementary Table 1, available online). This isotope study was carried out alongside a weed survey of the same fields. Table 1 gives details of the climate, manuring and irrigation regimes, summarized by farm. Since these are not experimental plots, better control over manure and water inputs was not possible, but these agricultural conditions represent real-life situations, providing an insight into the constraints imposed on agricultural decision making by the environment. No commercial N-containing fertilizers were used on any of the plots included in this study and had not been used for at least three years prior to crop sampling. For the most part, interviews were conducted with farmers who tended the fields. The mean annual rainfall quoted is from the Aquastat climate information tool (<http://www.fao.org/nr/water/aquastat/climateinfotool/index.stm>) from the Food and Agriculture Organization of the United Nations. The data for the Aquastat climate information tool originated from the CRU CL 2.0 data set (New et al., 2002) and use the means of observations made from 1961 to 1990. The aridity index (a numerical indicator of the degree of dryness) for each location was calculated by dividing mean annual precipitation by reference evapotranspiration (Middleton and Thomas, 1997), as quoted by the Aquastat climate information tool. Ten ears were randomly selected from each field, or were selected from five locations along a transect through each field. Half of the collected ears were then threshed, and a random selection of 50

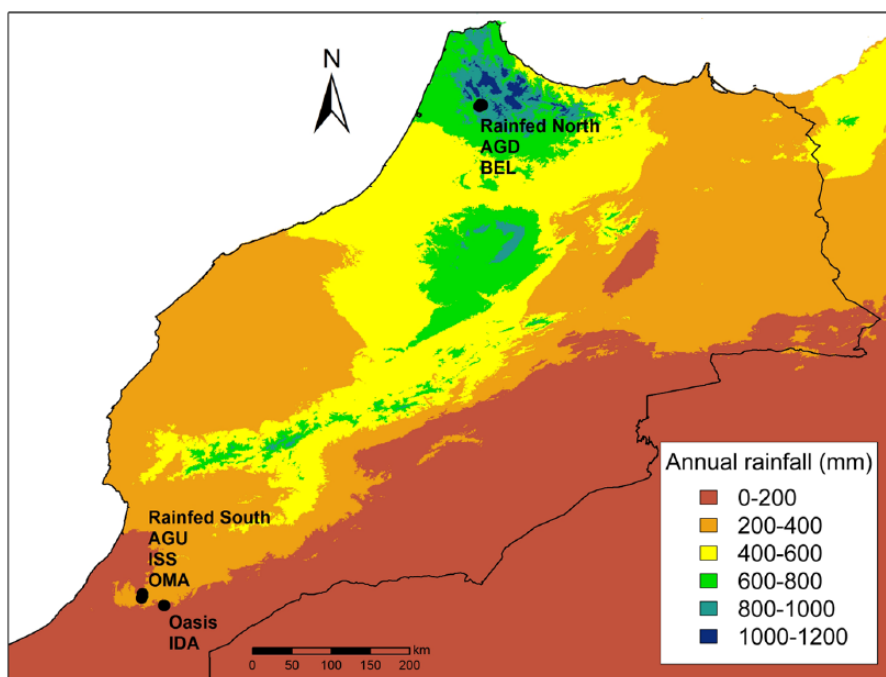


Figure 1. Rainfall map of Morocco with sampling locations marked. See Table 1 for explanation of site abbreviations.

grains was homogenized in a freezer mill to give an average isotope value for the growing condition. More details of the analytical methods are given in Supplementary Information, available online.

Figure 2 plots barley grain $\delta^{15}\text{N}$ values against $\Delta^{13}\text{C}$ values. Watering bands are defined from those determined for modern two-row hulled barley grains grown under different irrigation regimes in Borja, Spain (Wallace et al., 2013), and adjusted for the difference in $\Delta^{13}\text{C}$ values of two-row and six-row barley growing in the same watering conditions (c. 1‰; Voltas et al., 1998, 1999). The barley sampled in the Rif region of northern Morocco (Figure 1; AGD and BEL) received no manure and relatively high annual rainfall (703 mm/yr) and growing conditions are classified as dry-subhumid from the calculated aridity index (0.58; UNESCO, 1979). Its $\Delta^{13}\text{C}$ values fall into the well-watered band (Figure 2) and its relatively low $\delta^{15}\text{N}$ values, ranging from -0.3 to 1.4 ‰, plot within the no/low manuring band established from modern experimental farms in temperate Europe (Figure 3; Bogaard et al., 2013; Fraser et al., 2011).

Also plotted in Figure 3 are the $\delta^{15}\text{N}$ values of bread wheat grains grown in controlled conditions on newly established plots at an experimental station near Aleppo, Syria (Figure 5 and Supplementary Table 1, available online). These bread wheat plots received differing quantities of manure and irrigation water in two separate years of cultivation (see Fraser et al., 2011). There is no difference in the $\delta^{15}\text{N}$ values of bread wheat and barley grown under the same manuring conditions (Fraser et al., 2011), so barley and bread wheat $\delta^{15}\text{N}$ values can be used interchangeably here. As Fraser et al. (2011) note, the effect of manuring on cereal grain $\delta^{15}\text{N}$ values is variable on these newly established plots, with grain $\delta^{15}\text{N}$ values increasing significantly with manuring in the year when rainfall was higher (2009) but to a much lesser extent when rainfall was low. The level of

Table 1. Details of climate and manuring regimes at modern farming plots in Morocco, summarized by farm.

Farm	Commune and Province	Location (latitude, longitude)	Soil type	Annual rainfall (mm) ^a	Evaporation Potential (mm/month) ^a	Category	Manuring treatment	Collection date	Irrigation treatment
Agda (AGD)	Brikcha, Ouezzane	34.94, -5.58	Relatively deep, coarse brown soils on schist	703	1202	Rainfed north	None	May 2014	None
Bellota (BEL)		34.95, -5.54		703	1202	Rainfed north	None	May 2014	None
Agni Ouram (AGU)	Tighrit, Sidi Ifni	29.32, -9.44	Shallow, stony and rich in calcium carbonate (lithosols)	272	1426	Rainfed south	Biennially, if manure is available	Mar 2014	None
Issili Zemmouren (ISS)		29.35, -9.43		272	1426	Rainfed south	Biennially, if manure is available	Mar 2014	None
Id Lahso Omar (OMA)		29.38, -9.43		272	1425	Rainfed south	Biennially, if manure is available	Mar 2014	None
Id Aissa (IDA)	Amtoudi, Guelmin	29.24, -9.19	Relatively deep coarse-very coarse soils, formed by fluvial deposition. Dark in colour	194	1555	Irrigated	Added at sowing time (twice a year). Equivalent to c. 100 t/ha	Mar 2014	28 times a year to point of soil saturation

Note: ^aData from Aquastat (<http://www.fao.org/nr/water/aquastat/climateinfo/tool/index.stm>).

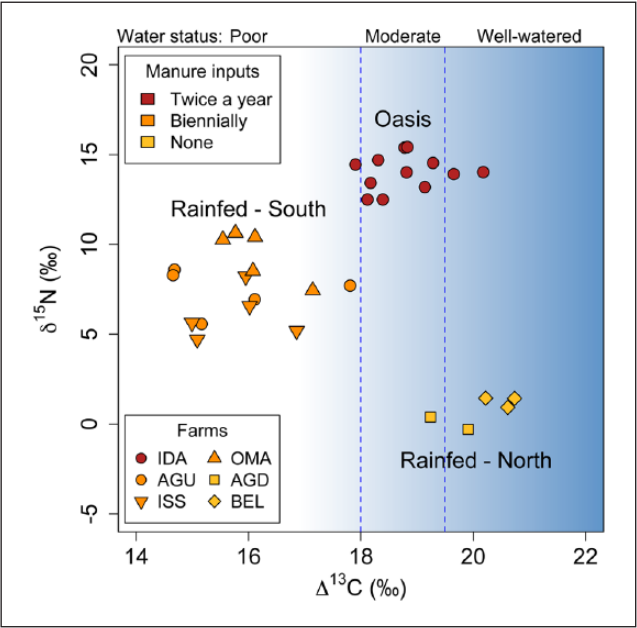


Figure 2. Plot of modern barley grain $\delta^{15}\text{N}$ against $\Delta^{13}\text{C}$ values, with watering bands for six-row barley marked.

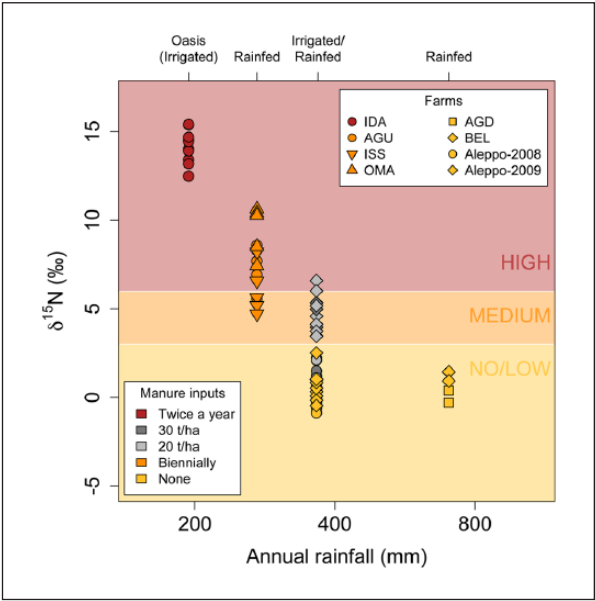


Figure 3. Plot of barley and bread wheat $\delta^{15}\text{N}$ values against a natural log scale of mean annual rainfall for their location. Manuring bands established from $\delta^{15}\text{N}$ values of cereals grown in experimental and traditional farming plots in temperate Europe are shown. NB. Aleppo plots were newly established in both years and therefore the effect of manuring on bread wheat $\delta^{15}\text{N}$ values was variable (grey symbols).

irrigation had no clear effect on bread wheat $\delta^{15}\text{N}$ values (Fraser et al., 2011). These results demonstrate that the effect of manure on crop $\delta^{15}\text{N}$ values can be detected after the first year of manure application, but the extent to which $\delta^{15}\text{N}$ values increase is variable, likely influenced by changes in annual rainfall and past land use.

The barley sampled in and around the Amtoudi oasis in southern Morocco (Figure 1) came from both rainfed and irrigated oasis fields. The rainfed fields are associated with three different farms (AGU, ISS, OMA) situated within 30 km of the oasis, but located at a higher elevation (up to c. 1110 m a.s.l. compared with c. 780 m a.s.l.), which means that they receive more rainfall (272 mm/yr compared with 194 mm/yr) and can support rainfed crops. Nevertheless, the growing conditions are classified as arid by the calculated aridity index (0.19; UNESCO, 1979). The rainfed fields received low quantities of sheep manure every two to three years. The barley $\delta^{15}\text{N}$ values range from 4.7 to 10.6‰ (Figure 2), the large variability likely reflecting a wide geographical region and therefore more varied environmental conditions, and considerable variation in manuring practice between fields. The barley $\Delta^{13}\text{C}$ values (Figure 2) indicate that they were relatively poorly watered, and thus that water was a limitation to growth. Figure 3 shows that the determined grain $\delta^{15}\text{N}$ values *overestimate* the level of manuring based on bands established in temperate Europe, since the majority fall within the high manuring band.

The fields in the oasis receive lower annual rainfall than the rainfed fields nearby, but they are irrigated every 15 days in autumn and every 7 days in summer (a total of 28 times over the growing season), by flooding to the point of saturation with water from an underground aquifer. It has been calculated that irrigation water input equates to c. 70 mm per irrigation cycle and c. 2000 mm/yr (see Supplementary Information for calculations, available online). The environmental conditions are classified as arid from the calculated aridity index (0.12; UNESCO, 1979), but if irrigation water input is also considered, the calculated aridity index is 1.42. The oasis fields receive sheep manure twice a year, with each autumn- and summer-sown crop, equating to c. 100 t/ha – an extremely high level of manuring that is readily observable in the dark colour and texture of the resulting artificial ‘dung-soil’ (see Table 1). The barley $\delta^{15}\text{N}$ values range from 12.5 to 15.4‰ (Figure 2; IDA), the smaller variation in $\delta^{15}\text{N}$ values compared with the rainfed fields reflecting a much more uniform method of manuring. The barley $\Delta^{13}\text{C}$ values (Figure 2) indicate that they were moderately to well-watered, demonstrating that irrigation inputs were sufficient to prevent water being a limitation to growth. In Figures 3 and 4, the $\delta^{15}\text{N}$ values of the barley from the oasis fields have been plotted against a natural log scale of mean annual rainfall for their location. We use mean annual rainfall rather than water input (i.e. rainfall + irrigation water) because it acts as an index of wider environmental conditions (e.g. temperature, evaporation potential), which all affect the ‘openness’ of the N cycle and thus the volatilization of ^{15}N -depleted N, resulting in higher plant $\delta^{15}\text{N}$ values. Moreover, the level of irrigation (water input ranging from c. 250 mm/yr to c. 1200 mm/yr on experimental plots near Aleppo) has not been found to affect cereal grain $\delta^{15}\text{N}$ values in previous studies (Fraser et al., 2011). The $\delta^{15}\text{N}$ values of the barley growing in the oasis fields plot correctly within the high manuring level band in Figure 3, but are *much* higher than the $\delta^{15}\text{N}$ values of any other cereal grains determined in temperate Europe (Fraser et al., 2011; Kanstrup et al., 2011).

Disentangling the effect of manuring from aridity on crop $\delta^{15}\text{N}$ values

Ideally, we would have sampled both manured and unmanured crops from all regions in Morocco in order to fully assess the difference in $\delta^{15}\text{N}$ values caused by manuring. However, no fields in the Rif region studied were manured because of a lack of available manure, and all fields in and around

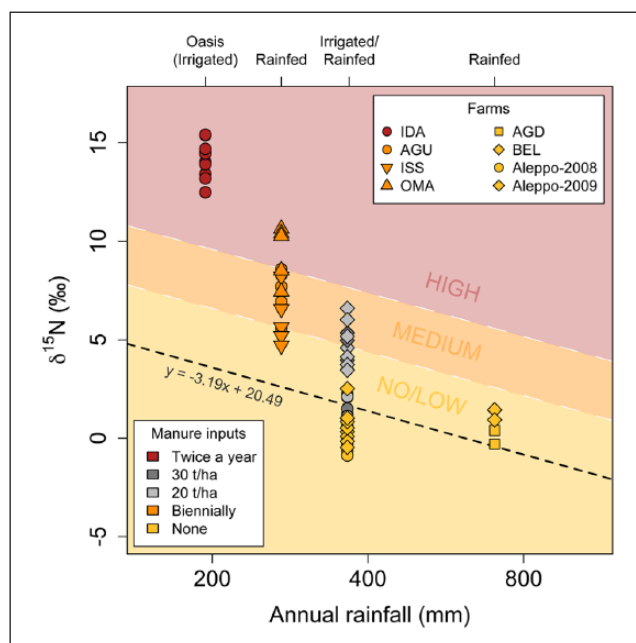


Figure 4. Plot of barley and bread wheat $\delta^{15}\text{N}$ values against a natural log scale of rainfall, with manuring bands adjusted for the regression between unmanaged plant $\delta^{15}\text{N}$ values and rainfall.

the Amtoudi oasis received some manure input. We have therefore made use of the trend determined by Hartman and Danin (2010) between $\delta^{15}\text{N}$ values of unmanaged (unmanured) annual plants and the natural log scale of mean annual rainfall in order to estimate the expected $\delta^{15}\text{N}$ values of unmanured cereals at different rainfall levels.

In the Hartman and Danin study, unmanaged plants were sampled along a rainfall transect in Israel, from 1000 mm to less than 50 mm mean annual rainfall. The regression for annual plants ($\delta^{15}\text{N} = -3.19[\log(\text{mean annual rainfall})] + 19.09$) from this study was chosen as the closest comparison to cereals. Furthermore, it ignores the $\delta^{15}\text{N}$ values of plants growing on exposed ridges, where very low N availability and reliance on atmospheric N-fixation by cyanobacteria leads to very low $\delta^{15}\text{N}$ values in areas of low rainfall (Evans and Ehleringer, 1993; West, 1990). Such conditions are unlikely to be relevant to arable land. More $\delta^{15}\text{N}$ data from unmanaged cereal grains growing in different rainfall regimes is needed to refine this relationship further, but this is difficult to attain since agriculture in many (semi-)arid regions now involves irrigation and/or artificial fertilizers and many of the soils have become too degraded for viable crop yields. In order to relate the $\delta^{15}\text{N}$ values of annual plant *leaves* determined in the Hartman and Danin study to the $\delta^{15}\text{N}$ values of cereal *grains*, the mean offset in $\delta^{15}\text{N}$ between bread wheat grains and leaves from a range of inorganic-N and manure fertilized plots throughout a growing season ($1.4 \pm 0.9\%$; Rothamsted Research, unpublished data, 1991) was used to adjust the regression line.

In Figure 4, we again plot the barley $\delta^{15}\text{N}$ values against the natural log scale of mean annual rainfall. We have used the regression between unmanaged plant $\delta^{15}\text{N}$ values, adjusted for grain-leaf offset ($\delta^{15}\text{N} = -3.19[\log(\text{mean annual rainfall})] + 20.49$), and the natural log scale of mean annual rainfall to adjust the manuring level bands established in temperate Europe. With these adjusted manuring bands, the $\delta^{15}\text{N}$ values of the sampled barley grains more accurately reflect the true manuring levels. The $\delta^{15}\text{N}$ values of barley grains from northern Morocco plot on the regression

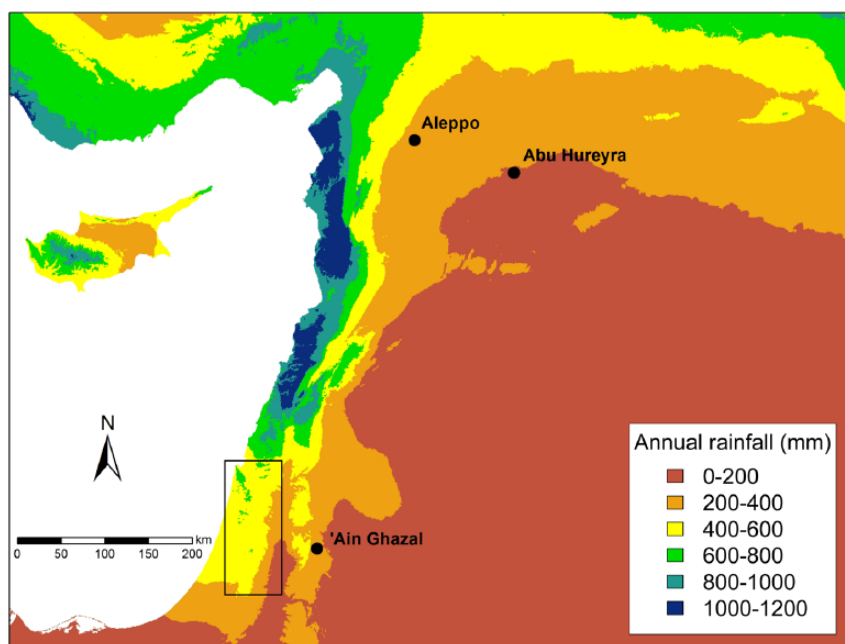


Figure 5. Map showing location of the archaeological sites Abu Hureyra and 'Ain Ghazal and the modern experimental station near Aleppo. Present-day annual rainfall is represented by shading. The area of the Hartman and Danin (2010) study is indicated by the box.

line for no manure, the $\delta^{15}\text{N}$ values of barley from the rainfed fields in southern Morocco plot around the medium level of manure, and the $\delta^{15}\text{N}$ values of barley from the oasis fields plot in the high manuring level band. The bread wheat grains from Aleppo also plot within the no/low and medium manuring level bands, which broadly corresponds to the rates of manure applied on these newly established plots. As noted above, manuring of plots in the drier year (2008) at Aleppo did not result in a clear increase in crop $\delta^{15}\text{N}$ values.

The adjusted manuring bands more accurately reflect known manuring practices in Morocco and Syria and can therefore tentatively be used to assess past manuring practice from crop $\delta^{15}\text{N}$ values at sites situated in areas with low rainfall. Herbivore bone collagen $\delta^{15}\text{N}$ values can also be used to estimate unmanured plant $\delta^{15}\text{N}$ values and therefore further ground truth this model on a range of archaeological sites.

The early farming sites of Abu Hureyra and 'Ain Ghazal

To date, the earliest evidence for crop cultivation has been found in southwest Asia, dating to the 10th millennium BC (Willcox, 2013). While much research has focused on determining the location and date of the earliest crop cultivation and subsequent domestication (e.g. Brown et al., 2009; Fuller et al., 2005; White and Denham, 2007; Zohary et al., 2012), less is known about the farming practices involved in this early agriculture. Isotopic analysis of the cereal grains themselves can reveal manure inputs ($\delta^{15}\text{N}$) and the water status of the crops ($\delta^{13}\text{C}$). To this end, this study determines $\delta^{15}\text{N}$ values of barley grain and pulse seed samples from the Pre-Pottery Neolithic B and Pottery Neolithic periods (PPNB and PN; c. 8800–6000 cal. BC) of Abu Hureyra and 'Ain Ghazal,

focusing on a period when domestic crop cultivation and animal husbandry were newly established and potentially integrated (Bogaard, 2005). Each sample represents between 5 and 80 individual grains/seeds, homogenized prior to analysis. The $\delta^{13}\text{C}$ values of the same barley grain and pulse seed samples have been determined by Wallace et al. (2015). No faunal isotope data are available from Abu Hureyra because of poor preservation of bone collagen, but $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of caprine bone collagen ($n = 50$) from PPN 'Ain Ghazal have also been determined previously (Makarewicz, 2007; Miller, 2012). This provides an unmanaged plant $\delta^{15}\text{N}$ baseline with which to compare the $\delta^{15}\text{N}$ values of the crop remains determined in this study. The results of these earlier isotope studies are summarized in the section 'Isotopic composition of fauna and crops from Abu Hureyra and 'Ain Ghazal'.

Abu Hureyra, on the southern bank of the Euphrates river (Figure 5), is a significant site as its occupation spanned the transition from a hunter-forager to farming economy. The site is located at the boundary of two contrasting environmental zones: drier steppe to the south and moist, often waterlogged, floodplain to the north (Moore et al., 2000: 3). Today the site receives c. 230 mm of annual rainfall but it has been estimated from $\delta^{18}\text{O}$ values of speleothems in the Soreq Cave that annual rainfall was c. 175–450 mm higher between 10,000 and 7000 BP (c. 8000–5000 cal. BC) than it is today (Bar-Matthews et al., 1997). We therefore plot the barley $\delta^{15}\text{N}$ values against a range of annual rainfall values between 175 and 450 mm higher than today's rainfall. In this study we focus on Abu Hureyra 2 (c. 8800–6000 cal. BC), the second phase of occupation, where cultivation of domestic crops and herding of domestic animals were the dominant means of subsistence for the sedentary community, although in the earliest period hunting of wild animals was still economically important. Hulled two-row barley grain samples ($n = 6$) covering all three periods of the site's occupation – 2A, 2B and 2C – were selected for isotope analysis.

Period 2A dates from c. 8800 to 7400 cal. BC, when the village encompassed c. 8 ha and large numbers of gazelle were hunted for meat alongside domestic crop cultivation and caprine husbandry (Legge and Rowley-Conwy, 2000). The crops grown comprise five domesticated cereals – rye, einkorn, emmer, bread wheat, two- and six-row barley – and at least three pulses – lentils, peas, vetches (De Moulins, 1997). Since both cereals and pulses were major crops, it has been surmised that crop rotation was practised to replenish the soil nutrients (De Moulins, 1997). Cultivation on the Euphrates floodplain has been dismissed by archaeobotanists working on the site since early cereals are believed to have been autumn-germinating (Hillman, 2000; Jones et al., 2013; Miller, 1987) and thus the spring floods caused by snow melt would have washed away the crops before harvest. Wadi sides, breaks of slope and wadi bottoms not prone to flooding could have offered areas for cultivation with enhanced soil moisture but reduced risk of flood damage (cf. Hillman, 2000). On the other hand, relatively high precipitation levels in the Early Neolithic suggested by the Soreq Cave speleothem data (Bar-Matthews et al., 1997) may have broadened potential cultivation zones beyond these restricted areas and into the steppe. Weed seeds recovered in the archaeobotanical assemblage are associated with cultivation on the drier steppe (De Moulins, 1997), although small-seeded legumes may have been derived from burning of dung.

In period 2B (c. 7400 to 6200 cal. BC) the site grew to c. 16 ha, with an estimated population of 5000 to 6000 people (Moore, 2000), although it has been argued that these figures have to be regarded as maximum limits, since shifting of the settlement cannot be excluded (Hole, 2000). Focus shifted from the hunting of gazelle to the adoption of large-scale herding of sheep and goats and the first significant exploitation of cattle (Legge and Rowley-Conwy, 2000). An increase in domesticated cereals and high proportions of weed seeds attest to the continued importance of crop cultivation (De Moulins, 1997). Pottery use began at the end of this period. Period 2C (c. 6200 to 6000 cal. BC) is the beginning of the Pottery Neolithic and saw the continuation of caprine

exploitation, with cattle and pig husbandry becoming more important. The ubiquity of legumes decreased in this period and it has been tentatively suggested that manure may have been used to maintain soil fertility, replacing the role of the small-seeded legumes in rotation with cereals (De Moulins, 1997). The nature of settlement also changed, with buildings spaced further apart and pits dug between them (Moore, 2000). The site contracted to c. 8 ha and at the end of this period was abandoned.

‘Ain Ghazal is located in modern Jordan (Figure 5). It is situated immediately adjacent to the Wadi Zarga, which would have been a permanent stream during the site’s occupation, in a transition zone between oak woodland and open steppe desert (Simmons et al., 1988). Today the site receives c. 250 mm of annual rainfall, placing it in an area vulnerable to fluctuations in rainfall. Analysis of wood charcoal found at the site has indicated the presence of deciduous oak woodland in the surroundings, which requires >300 mm rainfall per year and this has been used to infer that rainfall must have been at least 100 mm higher during the PPN (Neef, 2004). Again, the barley $\delta^{15}\text{N}$ values are plotted against a range of rainfall values between 175 and 450 mm higher than today’s rainfall (cf. Bar-Matthews et al., 1997).

‘Ain Ghazal was occupied for over 2000 years, from c. 8500 to 5500 cal. BC (Rollefson, 2015). The archaeobotanical samples included in this study comprise two-row hulled barley grain ($n = 9$) and pulse seed ($n = 7$) samples, spanning the site’s occupation during the Middle and Late PPNB (MPPNB and LPPNB; 8500–6900 cal. BC), which coincides with the Period 2A and 2B Abu Hureyra samples. In the MPPNB, herded sheep and goat accounted for the majority of the faunal assemblage, but there is evidence that a wide variety of wild animals were also hunted (Von den Driesch and Wodtke, 1997; Wasse, 2002). Domesticated cereals and a relatively high proportion of pulses were found in the archaeobotanical assemblage, with domestic two-row hulled barley, emmer and einkorn wheat, peas, lentils and chickpeas found in order of decreasing frequency (Neef, 1997). Sickles accounted for nearly 10% of the chipped stone tool inventory (Rollefson and Simmons, 1988), suggesting the economic importance of crop cultivation. By the beginning of the Late PPNB the site was c. 4–5 ha, but expanded to around twice that size by c. 7000 cal. BC (Köhler-Rollefson and Rollefson, 1990).

‘Ain Ghazal, unlike many sites in the Levant that were abandoned at the end of the PPNB, was occupied into the Yarmoukian phase of the Pottery Neolithic. Nevertheless, from the beginning of the PPNC, the variety of faunal species exploited by the inhabitants of ‘Ain Ghazal decreased significantly and it has been proposed that this was related to degradation of the surrounding landscape by removal of the natural vegetation through cultivation, grazing and deforestation (Köhler-Rollefson, 1988), exacerbated by climatic deterioration in c. 6200–6000 cal. BC (Bar-Matthews et al., 1999). Although the archaeobotanical samples in this study date to the Middle and Late PPNB, when evidence for landscape degradation is not as advanced as that in the later PPNC, there is the possibility that vegetation and accompanying soil degradation would have impacted the methods of cultivation, or indeed led to adaptations in order to redress the negative impacts of soil erosion on productivity.

To summarize, $\delta^{15}\text{N}$ values of hulled barley grain and pulse seed samples from the Early Neolithic sites of Abu Hureyra and ‘Ain Ghazal in the eastern Mediterranean were determined. These derive from both storage and refuse contexts spanning the Middle to Late PPNB and into the early PN, when crop cultivation was likely a mainstay of subsistence but reliance on hunting changed through time. Situated in areas where fluctuations in rainfall could have presented a risk to the success of crop cultivation, these sites are a good representation of cultivation in a relatively dry region. By placing the crop isotope determinations of barley from these archaeological sites in the framework of our modern study, we hope to constrain interpretation of cultivation practices, in

particular water management (considered in Wallace et al., 2015) and manuring, and thus reveal more about the nature and intensity of early agriculture.

Isotopic composition of fauna and crops from Abu Hureyra and 'Ain Ghazal

The $\delta^{15}\text{N}$ values of archaeological barley grain and pulse seed samples are given in Supplementary Table 2, available online, and their corresponding $\delta^{13}\text{C}$ and $\Delta^{13}\text{C}$ values, as well as the $\delta^{13}\text{C}_{\text{air}}$ values used to calculate the $\Delta^{13}\text{C}$ values, can be found in Wallace et al. (2015). As explained in the section 'Crop carbon isotope values', it is common practice to convert plant $\delta^{13}\text{C}$ values to $\Delta^{13}\text{C}$ values, to allow comparison between time periods when atmospheric CO_2 differed in its isotopic composition.

The determined barley grain $\Delta^{13}\text{C}$ values from Abu Hureyra are relatively varied (15.3–18.7‰; Figure 6), with samples falling predominantly in the moderate- to poorly watered band, with the exception of a well-watered outlier with $\Delta^{13}\text{C} = 18.7\text{‰}$ (Wallace et al., 2015). The large variation suggests that barley was cultivated on soils receiving variable water inputs and/or with varying water retention capacity. Although the crop isotope results cannot preclude floodplain cultivation (the relatively high $\delta^{15}\text{N}$ values could result from loss of ^{15}N -depleted nitrogen via denitrification as a result of seasonal flooding), given the risk posed to crops by spring floods, this variation in water status is more likely to be due to variation in the water retention of soils on the steppe or wadi sides. However, since the samples span the site's occupation, this variation could also be due to differences in annual rainfall over time (Wallace et al., 2015). The range in barley $\Delta^{13}\text{C}$ values at Abu Hureyra (3.5‰) is similar to that of the barley grown in rainfed fields in south Morocco (3.2‰; Figure 6), suggesting that variation could indeed have been due to such natural differences in the water retention of soils in the vicinity of the site.

The $\delta^{15}\text{N}$ values of barley grains from Abu Hureyra are relatively high, ranging from 6.4‰ to 10.4‰ (Figure 6). Without faunal $\delta^{15}\text{N}$ data it is not possible to estimate the $\delta^{15}\text{N}$ values of unmanaged plants in this region, but by plotting the Abu Hureyra barley $\delta^{15}\text{N}$ values against the natural log scale of the range of estimated past annual rainfall, it is possible to estimate the effect of aridity to some extent (Figure 7). The values plot within the high band of manuring at all annual rainfall values. There is no clear chronological difference in the $\delta^{15}\text{N}$ values and the low sample number precludes any statistical analysis. Some degree of manure application therefore cannot be excluded at Abu Hureyra during the PPNB and PN. However, it is possible that the relatively high crop $\delta^{15}\text{N}$ values were due to cultivation in soils that experienced temporary waterlogging and thus denitrification, such as on floodplains and wadi slopes (cf. Hartman and Danin, 2010). More work is needed to characterize crop growing conditions in such temporarily waterlogged soils and this should incorporate a multi-isotope approach, alongside weed ecological data, in order to distinguish different potential contributions to elevated crop $\delta^{15}\text{N}$ values (e.g. manuring and/or seasonal waterlogging).

The $\delta^{15}\text{N}$ values of these barley grains dating to the PPNB and PN are not significantly lower than those of individual rye grains (8.7–11.1‰; $p = 0.113$) dating to the Epipalaeolithic (determined by Araus et al., 2014), despite the >1500 year gap between their deposition. The authors have suggested that the high $\delta^{15}\text{N}$ values of these rye grains are due to them growing on 'dump-heaps', which likely had higher $\delta^{15}\text{N}$ values than the surrounding soils. The climate is also believed to have been drier than the succeeding PPN (Bar-Matthews et al., 1997). This demonstrates that if decreasing fertility of soils in the wider region was leading to decreasing crop $\delta^{15}\text{N}$ over time (cf. Araus et al., 2014), soil conditions were being managed in some way at Abu Hureyra in the PPNB, maintaining higher crop $\delta^{15}\text{N}$ values than those expected for unmanaged plants.

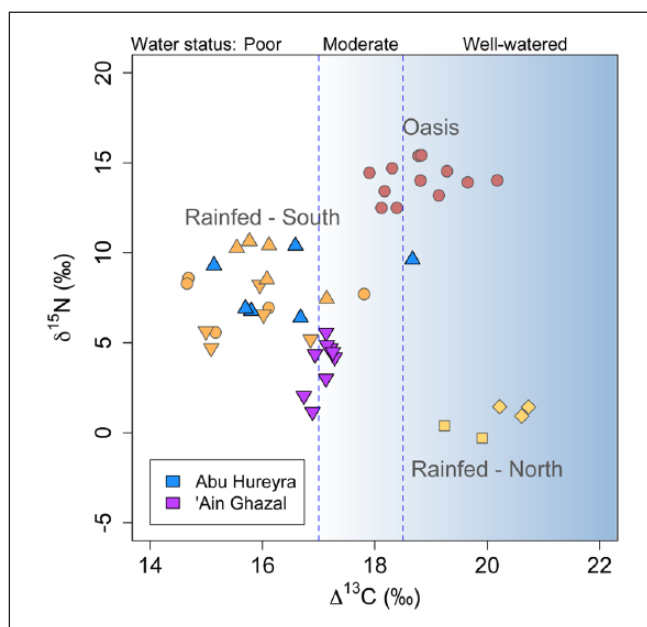


Figure 6. Plot of $\delta^{15}\text{N}$ against $\Delta^{13}\text{C}$ values of barley grain from Abu Hureyra and 'Ain Ghazal, with modern barley grain isotope values plotted for reference. The watering bands for 2-row rather than 6-row barley are marked, to allow for comparison with archaeological 2-row barley $\Delta^{13}\text{C}$ values.

The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of bone collagen from 36 goats, 4 sheep and 10 caprines from 'Ain Ghazal (MPPNB to PPNC) have been determined by Miller (2012) and Makarewicz (2007). The $\delta^{13}\text{C}$ values range from -20.7‰ to -16.2‰ and the $\delta^{15}\text{N}$ values range from 6.8‰ to 10.8‰ , with a mean of 8.9‰ . There was no statistical difference in $\delta^{15}\text{N}$ values between the chronological phases of the PPNB, but Miller observed that both the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of caprines from the PPNC ($n = 9$) were higher than the PPNB. She interprets this as evidence for greater consumption of C_4 plants growing in more arid environments (Miller, 2012). The inferred mean unmanaged plant $\delta^{15}\text{N}$ value estimated from the faunal bone collagen is 4.9‰ . This can be compared with the $\delta^{15}\text{N}$ values of the barley grains in this study and also to the regression line of $\delta^{15}\text{N}$ against natural log transformed rainfall determined from the Hartman and Danin (2010) study (Figure 7). The fact that the estimated faunal plant diet $\delta^{15}\text{N}$ value plots in the medium band of manuring suggests that the herbivores were consuming plants growing in areas of the landscape where loss of N was enhanced, perhaps in seasonally wet stream beds where moisture levels and therefore plants remain into the summer months (Hartman and Danin, 2010). This highlights the benefit of considering herbivore bone collagen $\delta^{15}\text{N}$ values in relation to the regression of plant $\delta^{15}\text{N}$ values and rainfall in order to identify potential feeding strategies that could bias the unmanaged plant $\delta^{15}\text{N}$ baseline value.

There is very little variation in the $\Delta^{13}\text{C}$ values of barley grain samples from 'Ain Ghazal (16.7‰ – 17.3‰ ; Figure 6; Wallace et al., 2015). This range is as tight as that determined in modern barley grown under the same water conditions (Wallace et al., 2013), suggesting that farmers at 'Ain Ghazal had strict control over water availability, possibly through the use of the spring and floodplains close to the site. Their $\Delta^{13}\text{C}$ values fall into the moderately watered band, again consistent with a degree of intentional watering to improve their water status. The floodplain at 'Ain Ghazal is very limited in extent and thus unlikely to have supported enough crop cultivation to provide the

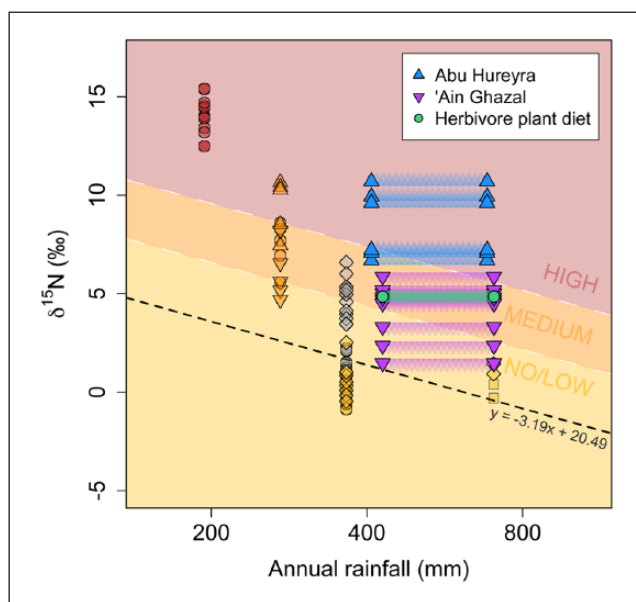


Figure 7. Plot of archaeological barley $\delta^{15}\text{N}$ values against a natural log scale of rainfall, with manuring bands adjusted for the regression between unmanaged plant $\delta^{15}\text{N}$ values and rainfall. The $\delta^{15}\text{N}$ values of modern barley and bread wheat grains from Morocco and Syria are plotted for reference.

population of the 5–10 ha settlement. It seems therefore that well-watered conditions were maintained elsewhere, possibly on terraced fields close to the spring. The $\delta^{15}\text{N}$ values of barley grains from 'Ain Ghazal are relatively low, ranging from 1.2‰ to 5.6‰ (Figure 6). The barley $\delta^{15}\text{N}$ values are similar to or lower than the unmanaged plant $\delta^{15}\text{N}$ baseline estimated from the faunal $\delta^{15}\text{N}$ data (4.9‰), suggesting that the barley was growing in soil with a lower $\delta^{15}\text{N}$ value than plants consumed by herbivores. When the 'Ain Ghazal barley samples are plotted against the natural log scale of the range of estimated past annual rainfall, they plot within the low-medium bands of manuring (Figure 7), implying that any manuring input was minimal.

The $\Delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of pulses from 'Ain Ghazal ($n = 7$) have also been determined (Supplementary Table 2, available online). The $\Delta^{13}\text{C}$ values are relatively varied and fall within the moderate- to poorly watered band (Wallace et al., 2015). The greater variation in pulse $\Delta^{13}\text{C}$ values compared with barley could be because pulses tend to be more sensitive than cereals to water shortages during seed filling (Brouwer et al., 1989), so variation in water availability during this growth period would result in greater variation in pulse compared with barley $\Delta^{13}\text{C}$ values. The pulse $\delta^{15}\text{N}$ values range from 0.9‰ to 2.3‰, which is close to but slightly higher than the $\delta^{15}\text{N}$ value of atmospheric-N (Yoneyama et al., 1986), and this indicates that they indeed received the majority of their N from N-fixation. The slight elevation of pulse seed $\delta^{15}\text{N}$ values over that of atmospheric-N could indicate some uptake of soil-N, which is more favourable when the concentration of soil inorganic-N is high (e.g. in manured soils; Streeter and Wong, 1988; Waterer and Vessey, 1993).

Thus we see evidence for two different strategies of crop cultivation in the PPNB and early PN, although greater numbers of crop samples for isotope analysis would be needed to characterize these more fully. At Abu Hureyra, barley was grown in rainfed fields whose soils varied in their water-holding capacity, with organic matter levels equivalent to high manuring rates, potentially

relating to dung from the domestic sheep and goats herded in the vicinity of the site. At 'Ain Ghazal, farmers seemed to have had strict control over the water status of their barley, possibly through watering their crops from the spring. There is no evidence for intensive manuring at the site, possibly because sheep and goats were herded away from the settlement (cf. Köhler-Rollefson and Rollefson, 1990), or periodic grazing of sheep and goats on arable fields did not result in high levels of manuring (cf. Bogaard and Isaakidou, 2010). The relatively high frequency of pulses recovered from the site could also reflect the practice of cereal–pulse rotation, which would have helped to replenish soil nutrients.

Conclusions

Although a relatively small modern pilot study, the $\delta^{15}\text{N}$ values of barley grains growing in fields in present-day Morocco have provided a framework for reconstructing past farming practices, in areas where a (semi-)arid environment complicates the manuring $\delta^{15}\text{N}$ signal. We use the regression line established by Hartman and Danin (2010) between unmanaged annual plant $\delta^{15}\text{N}$ values and the natural log of annual rainfall to assess the relative importance of manuring at two early farming sites. Taken together, the material evidence from archaeological sites and crop isotope values can constrain scenarios of early farming practice. For example, low crop $\delta^{15}\text{N}$ values preclude intensive manuring at 'Ain Ghazal, which is consistent with the theory that the high frequency of pulses at the site is linked to maintenance of soil fertility. By contrast, the relatively high $\delta^{15}\text{N}$ values of barley from Abu Hureyra cannot exclude the possibility of manuring as a means of maintaining soil fertility, although it is possible that cultivation of temporarily waterlogged soils (wadi bottoms and slopes) contributed to these high $\delta^{15}\text{N}$ values. In both situations, however, the importance of manuring inferred from crop $\delta^{15}\text{N}$ values would have been overestimated using the manuring level bands established for temperate Europe, rather than the framework developed here. This study provides the basis upon which to carry out further $\delta^{15}\text{N}$ value determinations of modern cereal grains at other locations – and particularly on floodplains – to refine this framework, further constraining interpretations of possible farming regimes. The emerging evidence for crop growing conditions and early farming practices in western Asia presented here, as in Neolithic regimes in Europe (e.g. Bogaard et al., 2013), suggests that the flexibility of the early farming 'package' of cereals, pulses and livestock enabled communities to develop contrasting strategies to adapt to different ecological and social settings.

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