Variations in the $^{13}C/^{12}C$ ratios of modern wheat grain, and implications for interpreting data from Bronze Age Assiros Toumba, Greece

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ABSTRACT

Variations in the $^{13}C/^{12}C$ ratios of wheat grain at different spatial and temporal scales are examined by analysis of modern samples, including harvests of einkorn and durum wheat from Greece, and serve as a guide to interpreting data for Bronze Age grains from Assiros Toumba. The normal distribution and low variability of $\delta^{13}C$ values of einkorn from 24 containers in the Assiros storerooms are consistent with pooling of local harvests, but less likely to represent the harvest of several years or include grain imported from further afield. Correlation between emmer and spelt $\delta^{13}C$ values provides strong support for other evidence that these were grown together as a maslin crop. ^{13}C discrimination (Δ) for the Bronze Age samples is estimated to be 2.5‰ larger than at present, and would be consistent with an intensive, horticultural regime of cereal cultivation, *possibly* involving some watering.

Keywords: Carbon isotopes; wheat; archaeobotany; Assiros Toumba.

1. Introduction

The $^{13}C/^{12}C$ ratio of carbon in plants depends on the composition of atmospheric CO_2, and a wide variety of factors which affect plant growth and influence the carbon isotope fractionation associated with photosynthesis (Farquhar et al., 1989). These factors include the local environmental conditions in which the plant grows: particularly the natural properties of the climate and soil affecting water availability, and any modifications resulting from cultivation. Because of this, $^{13}C/^{12}C$ analyses of the preserved remains of archaeological plants are becoming increasingly relevant to studies of the nature of past environmental conditions, the development of agriculture, and the dietary habits of ancient populations (Aguilera et al., 2008; Araus and Buxo, 1993; Araus et al., 1997; Araus et al., 2003; Araus et al., 2001; Ferrio et al., 2006; Ferrio et al., 2005; Heaton, 1999; Marino and DeNiro, 1987; Ogrinc and Budja, 2005; Riehl et al., 2008; Tieszen, 1991)

The conclusions drawn in such studies often involve interpretation of differences in $^{13}C/^{12}C$ ratios based on data for a limited number of samples, and a proper interpretation of such data must be founded on an appreciation of the significance of spatial or temporal differences in the $^{13}C/^{12}C$ ratios of plants (Codron et al., 2005; Heaton, 1999; Tieszen, 1991; Vanklinken et al., 1994). This understanding can only be derived from $^{13}C/^{12}C$ analysis of modern plants. However, although such analyses are widely used in botanical studies, very few studies of $^{13}C/^{12}C$ variations among modern plants have been specifically directed toward a better understanding of archaeological samples (Codron et al., 2005; Heaton, 1999).

Our interest in the spatial and temporal variations in wheat $^{13}C/^{12}C$ ratios was prompted by our analysis of a large number of samples of charred wheat grains from a Bronze Age tell site near the modern village of Assiros in N. Greece (Fig. 1). Wheat is a particularly important crop in the farming history of SW Asia and Europe and, where its grains are preserved by charring, $^{13}C/^{12}C$ analysis has been used as a basis for investigations of water availability, irrigation, and cereal productivity in prehistoric agriculture (Aguilera et al., 2008; Araus and Buxo, 1993; Araus et al., 2003; Araus et al., 2001; Ferrio et al., 2005). In this work we present data for the $^{13}C/^{12}C$ variations which may be found among samples of modern wheat grain, assess the likely effects of charring, and use the results as a basis for interpreting $^{13}C/^{12}C$ data for samples from Bronze Age Assiros Toumba.

1.1. Assiros Toumba

Excavations at Assiros Toumba revealed a number of storerooms burned to the ground about 1300 BC (Wardle, 1987; Wardle, 1988; Wardle, 1989). Large quantities of grain, originally held in clay bins, clay-lined baskets and large ceramic storage jars (*pithoi*), had been preserved by charring and burial beneath the collapsed roofs of the rooms. The containers held a variety of crop species including barley, broomcorn millet, bitter vetch, and several species of wheat: einkorn (*Triticum monococcum*), emmer (*T. dicoccum*), the 'new type' glume wheat, spelt (*T. spelta*) and, not distinguishable from one another, either bread wheat (*T. aestivum*) or durum wheat (*T. durum*) (Jones et al., 2000; Jones et al., 1986). The spelt and emmer were normally found together, and may have been grown together as a mixed crop, or 'maslin' (Jones, 1987).

The six excavated storerooms had a combined floor area of at least 90-100 m^2 that was entirely given over to storage of crops and, to date, no habitation space has been uncovered from this phase of the site's history. This remarkable concentration of food storage, which invites comparison with the much larger and much grander 'palatial' complexes of Bronze Age S. Greece, poses the question of whether this centralised storage was under elite control or represented a communal initiative (Andreou, 2001; Jones et al., 1986). A related question is whether it represents storage of a single year's harvest gathered from a wide geographical area – implying that the inhabitants of Assiros Toumba had far-reaching influence - and/or storage of more than one year's harvest – implying the holding back of surplus as a provision against subsequent crop failure.

Because $^{13}C/^{12}C$ ratios of plants are sensitive to environmental conditions, and such conditions are likely to differ in space and time, we postulated that wheat grain from different sites and different years might display different $^{13}C/^{12}C$ ratios. On this basis analysis of the $^{13}C/^{12}C$ ratios of the Assiros Toumba grain might provide information on the origin of the harvest/s.

2. Materials and methods

2.1. Modern samples

We present data for modern samples of wheat collected from three sources:

(1) whole plants of bread wheat (*Triticum aestivum ssp. vulgare* cv. 'Mercia') from field trial plots at Sutton Bonington, University of Nottingham, England, harvested in July 1997;

(2) ears of archived samples of einkorn (*T. monococcum*), emmer (*T. dicoccum*), and spelt (*T. spelta*) from the John Innes Centre (Norwich, England) harvested in 1981, 1985, 1992 and 1999 with the different species grown under identical conditions;

(3) grain of einkorn and of the Athos variety of durum wheat (*T. durum*), harvested between 1991 and 2002, from agricultural research stations at Thessaloniki and Agios Mamas in N. Greece, ca. 30 and 65 km from Assiros respectively; grain of the Athos variety of durum wheat, harvested in 1994 and 1995, from research stations in N., C. and S. Greece; and grain of the Apulo, Cosmodur and Simeto varieties of durum wheat,

harvested in 1995 and 1998, from individual farmers around Assiros and nearby villages (Fig. 1). The crops were all rain-fed, i.e. received no artificial irrigation.

2.2. Charring experiments

Each charring experiment involved placing a crucible with 20-30 grains of modern, air-dried einkorn in a hot oven. Two samples of einkorn were used, one from Greece and one from France, with charring at 210, 220, 240 and 250°C for 3 days open to the atmosphere (‘open’), or for 3 or 5 days closed to the atmosphere by wrapping in tin foil and burying in sand (‘closed’). These conditions were chosen on the basis of earlier charring experiments, which have shown that they provide the closest replication of those under which Bronze Age samples at Assiros Toumba were charred (unpublished data).

2.3. Bronze Age wheat from Assiros Toumba

Charred grain was recovered from the containers in the storeroom complex by flotation of their contents. Flotation samples were taken from the most dense concentration of plant remains within each container and also from the more mixed deposits between concentrations. Grain was submitted for isotopic analysis only from the purest, densest concentrations, the locations of which are shown in Fig. 2.

2.4. Analytical methods

Samples were oven dried at 50°C. Thorough grinding and homogenisation of modern grains is important, because the more resistant grain coats were found to have δ^{13}C values typically 1‰ lower than the softer interiors (see below). In the earliest part of the study individual whole grains were ground by hand in an agate mortar and pestle. The <250 micron and >250 micron portions were separated, weighed, analysed individually, and the whole grain δ^{13}C value calculated by mass balance. Later samples were whole ground and homogenised in a liquid N_2 freezer mill (SPEX). Charred samples were easily ground and homogenised by hand.

^{13}C/^{12}C ratio analyses were performed by combustion in a Carlo Erba NA1500 elemental analyser on-line to a VG TripleTrap and Optima dual-inlet mass spectrometer. The ratios are reported as δ^{13}C values in per mille (‰), where:

$$\delta^{13}C = \left(\frac{{}^{13}C/{}^{12}C_{sample}}{{}^{13}C/{}^{12}C_{reference}} - 1 \right) \times 10^3$$

with values calculated to the VPDB reference by comparison with co-run laboratory standards (of plant material) calibrated against NBS-19 and NBS-22. Replicate analysis of well-mixed samples indicated a precision of ± <0.08‰ (1 SD, n = 8). %C and %N were determined by reference to an acetanilide standard.

3. δ^{13}C variations in modern wheat grain

3.1. Variations within a harvest

The variation in the δ^{13}C values of grain from Mercia bread wheat (Sutton Bonington, England) at different spatial scales is shown in Table 1. Differences between grains from the same spikelets are generally small, about 0.1‰, only slightly higher than the standard deviation of the analytical precision. Grains from different spikelet positions on the same ear, however, displayed a progressive increase in δ^{13}C value toward the top of the ear, with a particularly large difference at the terminal spikelet (Fig. 3). The resulting within-ear standard deviations were about 0.4 and 0.6‰. Deviations of a similar magnitude, typically 0.3 to 0.7‰, are also found within single plants (different ears on the same plant), among different plants on the same plot, and among grains randomly sampled from single harvests (Table 1).

From the data in Table 1 it appears that much of the 0.3 to 0.7‰ variation in δ^{13}C values among individual grains of a particular harvest can be traced to variations within individual ears, reflecting the relatively uniform ripening for which domesticated cereals have been selected. The variation within individual ears probably reflects both the changing environmental conditions and variety of sources of carbon used during the growth of wheat grains (Abbad et al., 2004; Araus et al., 1992; Blum, 1998; Bort et al., 1996; Plaut et al., 2004). Much of the carbon may be derived from photosynthetic fixation occurring during grain filling, which utilises both atmospheric CO_2 and internally-respired CO_2. Under conditions of stress (e.g. reduced water availability, spreading foliar disease, onset of senescence) that tend to occur at this time, however, grain filling may also utilise earlier-assimilated carbon remobilised from the stem. With grains at different positions on the ear filling at slightly different times, temporal variations in these factors may be expected to cause variations in grain $\delta^{13}C$ values along the ear, with the least variation at the scale of the individual spikelet.

Variation in whole grain $\delta^{13}C$ values may also arise as a result of variations in composition. Crude separation of individual Mercia grains into different components showed that the coat-rich and embryo-rich portions of a grain had $\delta^{13}C$ values about 1‰ lower than the endosperm. This corresponds to the known tendency for biochemical components to fractionate $\delta^{13}C$ values in the order $\delta^{13}C_{\text{lipid}} < \delta^{13}C_{\text{protein}} < \delta^{13}C_{\text{carbohydrate}}$ (Dungait et al., 2008; Lichtfouse et al., 1995). It is not known to what extent variations in the proportions of the different components of a grain influenced whole grain $\delta^{13}C$ values in Table 1. Apart from grains on the terminal spikelets of Fig. 3, which had a lower mass than other grains, there was no clear relationship between grain size and $\delta^{13}C$ value. We note, however, that the chaff, which might be expected to be more compositionally uniform than the grain, did tend to show less variation in $\delta^{13}C$ along the ear (Fig. 3).

We may gauge the significance of these within harvest variations by assuming a typical 1 SD variation of 0.5‰. If two samples were to be compared, with each based on analysis of, for example, 5 grains, then a Students t-test would require the mean values of the samples to differ by at least 0.7‰ for them to be considered significantly different at the 95% confidence level. When differences of this magnitude (i.e. about 0.7‰) are found in studies of archaeological wheat, they can only be regarded as significant when they are based on much larger numbers of grains.

3.2. Differences between harvests

$\delta^{13}C$ values for durum and einkorn grain from harvests of different years and different locations in Greece are shown in Table 2, and Figs. 4 and 5. Inter-annual variations at Agios Mamas and Thessaloniki were highly variable, ranging up to 2.7‰ between some years, but with insignificant differences between other years (Fig. 4). Zhao et al. (2001) reported more modest inter-annual variations of 0 to about 1.3‰ for unfertilised wheat grain grown at Rothamsted, England, during the past 150 years, and found no clear relationship to rainfall. At semi-arid Agios Mamas and Thessaloniki, rainfall is more likely to be the limiting factor on cereal growth and so to influence the isotopic composition of grain, although these relationships are far from simple. Because winter rainfall is modest and unreliable in northern Greece, spring rainfall may be critical to grain filling, as indicated by a local saying that predicts a happy outcome for farmers 'if it rains twice in April and once in May'. Spring rainfall is less important after a wet winter, however, and its benefits may conversely be neutralised by desiccating early summer winds. Finally, in examining possible relationships between rainfall and $\delta^{13}C$ values, agricultural stations (such as Agios Mamas) emphasise that their weather data are far from infallible. More reliable readings may be available from distant meteorological stations, but these data are probably irrelevant in a region subject to acute local variation in rainfall.

There is nevertheless some suggestion that rainfall in May – i.e. in the weeks before typical mid-June to early July harvesting – could have an effect on durum grain $\delta^{13}C$ values at Agios Mamas. For six of the ten sampled years $\delta^{13}C$ values were between -25.6 and -24.8‰, with May rainfall between 17 and 59 mm (average 36 mm). In contrast, three of the four years having significantly higher $\delta^{13}C$ values (-23.9 to

-23.3‰), corresponded to a very dry May with ≤4 mm (1995, 1997, 2002). The fourth year with a high $\delta^{13}C$ value (2001) followed a very wet May (84 mm), but farmers in Assiros reported that this was offset by hot and desiccating southerly winds in May and the same may have happened at Agios Mamas. The $\delta^{13}C$ values from Thessaloniki are more difficult to interpret: a high value for 1997 followed the driest May of the decade and that for 1993, which followed a wet spring, might be attributed to desiccating winds that again caused problems in Assiros, but high values in 1998-99 cannot be explained on available climatic evidence.

Climate and other factors influencing water availability, such as soil condition, vary with location. Geographic differences in $\delta^{13}C$ must therefore also be anticipated. As expected, these are found to depend on the degree of spatial separation of the sites. Single varieties of durum wheat harvested in the same year from different fields around Assiros, and around neighbouring villages up to 10 km from Assiros, displayed a maximum difference in $\delta^{13}C$ values of only 1.3‰ (Fig. 5). In contrast, single varieties of durum from about 35 km apart (Agios Mamas and Thessaloniki) differed by up to 2.2‰ (Fig. 4), whilst those from locations several hundred kilometres apart differed by up to 3.2‰ (Fig. 5).

Samples having large differences in $\delta^{13}C$ values are therefore likely to reflect either harvests from widely separate locations, or harvests from different years. However, such spatial or temporal differences are not precluded where samples have essentially identical $\delta^{13}C$ values: in 1995 durum wheat from Assiros had $\delta^{13}C$ values close to those of durum from Tripoli in southern Greece (Fig. 5), and harvests from different years can have very similar $\delta^{13}C$ values (Fig. 4).

3.3. Differences between species/varieties

Data for einkorn, emmer and spelt grown under identical conditions (within each year) at the John Innes Centre are shown in Table 3. In each year spelt had the highest $\delta^{13}C$ values, between 2.1 and 3.4‰ higher than those of einkorn. In some years emmer had $\delta^{13}C$ values 1.0 to 1.4‰ higher than those of einkorn, in other years there was little difference between emmer and einkorn. Differences in $\delta^{13}C$ are also evident between durum and einkorn grown at Agios Mamas, with einkorn having the higher values (Fig. 4).

Whilst these patterns are observed in comparisons of different species, variations in $\delta^{13}C$ might equally be anticipated among different varieties of the same species. Thus, whilst we observed no clear distinction between the Cosmodor and Simeto varieties of durum wheat grown near Assiros in 1998 (Fig. 5), detailed trials of durum genotypes grown under Mediterranean conditions have been found to yield grain $\delta^{13}C$ values differing by up to 3.4‰ between different genotypes (Merah et al., 2001). The differences between species described above may therefore not be consistent, but could equally well be accounted for by chance differences due to the varieties sampled.

3.4. Effect of charring on $\delta^{13}C$ values

The effects of charring are shown in Figure 6, where it is apparent that the different durations (3 or 5 days) or conditions (open versus closed) of charring had little influence; the main differences being observed between the two sources of einkorn. For the Greek sample (Fig. 6a), charring resulted in a slight reduction in C/N ratios, but little overall change in δ^{13}C values. In contrast, charring of the French einkorn (Fig. 6b), which started with a much higher C/N ratio, resulted in a more marked reduction in C/N ratio and an increase in δ^{13}C values of about 0.8‰.

An increase in δ^{13}C values could result from the preferential loss of lipids, which tend to have δ^{13}C values lower than other components (Lichtfouse et al., 1995). As lipids contain little nitrogen, their preferential loss should result in a decrease in C/N ratio, as is observed in Fig. 6, and is also evident in the data reported by Aguilera et al. (2008). ^{15}N/^{14}N ratios of einkorn appear to be little affected by charring (Bogaard et al., 2007).

Previous experiments on the effect of charring on the δ^{13}C values of wheat grain were carried out at higher temperatures and over shorter durations than those used in this study. Carbonisation of grains of durum wheat, at temperatures reaching 400°C or 600°C for up to 2 hours, resulted in δ^{13}C values increasing by up to about 1‰ (Araus and Buxo, 1993; Araus et al., 1997), whereas bread wheat carbonised for short periods at 250°C showed negligible change in δ^{13}C (Ferrio et al., 2007). Within the constraints of these experiments, the effect of charring on the δ^{13}C values of wheat is generally ignored (Araus et al., 2003; Ferrio et al., 2007).

4. Assiros Toumba

4.1. The identification of maslin

δ^{13}C values for the Bronze Age wheat samples from Assiros Toumba are listed in Table 4. Each sample value represents the average of 5 grains. For those samples where the grains were analysed individually the average standard deviation was 0.45‰ (Table 4), which is similar to that found among single field harvests of modern wheat grain (Table 1).

The distribution of $\delta^{13}C$ values for samples of different species is displayed as histograms in Fig. 7. It is clear that, whereas emmer and spelt were not distinguishable from one another, the mean value for einkorn is significantly lower than the means for emmer and spelt (Fig. 7). Whilst direct comparison between ancient and modern varieties must be treated with caution, it is clear from the modern data (Table 3) that the different species can exhibit large differences in $\delta^{13}C$ even when grown under the same conditions, and so the difference in $\delta^{13}C$ values between einkorn and the other wheat species at Assiros Toumba need not reflect different growing locations or years.

In most parts of the storeroom complex, emmer and spelt were found in association with one another, and it has been suggested that they were grown together as a mixed crop, or 'maslin' (Jones, 1987). Although the mean $\delta^{13}C$ values for emmer and spelt are very similar, there is a total range of about 1.5‰. A plot of the $\delta^{13}C$ values of paired samples taken from the same containers demonstrates that the emmer and spelt $\delta^{13}C$ values are well-correlated across this range (Fig. 8), the Spearman's Rank Correlation Coefficient of 0.75 being significant at 99% confidence. This lends support to the interpretation of these samples as representing intentional emmer/spelt maslins rather than unintended mixing of the two species during the destruction of the storerooms or subsequent salvage efforts. The slight differences in the $\delta^{13}C$ values of maslin mixtures from different containers may be the result of each container holding wheat harvested from a different field.

4.2. Spatial and temporal distribution of cultivation

The $\delta^{13}C$ data for the wheat at Assiros Toumba show no evidence for significant temporal or spatial differences in harvests. The distributions of $\delta^{13}C$ values for the three species appear to be normal (at least for the more abundant einkorn, Fig. 7), and the ranges of values – 1.2‰ (einkorn), 1.2‰ (emmer) and 1.4‰ (spelt) – are in fact very similar to the 1.3‰ range for modern durum wheat harvested in one year from farms close to Assiros (Fig. 5). The narrowness of these ranges means that there is little evidence for any significant differences between the individual samples of a particular species. Thus it was noted above that samples would need to differ by at least 0.7‰ to be considered significantly different at the 95% confidence level. For Assiros, 18 of the 24 einkorn samples fall within this 0.7‰ range, and therefore have $\delta^{13}C$ which are statistically indistinguishable. In addition, no particular characteristics distinguished the six remaining samples having the highest or lowest $\delta^{13}C$ values.

In all respects the data are therefore *consistent* with the different samples of wheat at Assiros Toumba having been harvested in the same year and from a limited geographic area. From our knowledge of variations among modern wheat harvests, however, we cannot absolutely preclude the possibility that the wheat in the Assiros Toumba storerooms represents harvests from different years or locations which, coincidentally, have identical $\delta^{13}C$ values.

4.3. Crop water status

In terms of absolute values, it is instructive to compare the composition of the Bronze Age einkorn from Assiros Toumba with modern samples of the same species from the same area. Einkorn is no longer cultivated at Assiros, but we estimate that its hypothetical modern value would be about –22.4‰ on the basis that:

(1) the average δ^{13}C value for einkorn at Agios Mamas during 1991-1998 was -23.2‰;

(2) the differences between the δ^{13}C values of durum grown at Agios Mamas and at the different farms around Assiros averaged 0.8‰ (in both 1995 and 1998).

To compare this modern value of -22.4‰ to the mean value of -23.5‰ for the Bronze Age einkorn (Fig. 7), we must take into account known historic changes in the isotopic composition of atmospheric CO_2. This is done by expressing the wheat isotope composition relative to atmospheric CO_2 using the value for discrimination, Δ, where:

$$\Delta_{plant} = \frac{\delta^{13}C_{air} - \delta^{13}C_{plant}}{\left(1 + \delta^{13}C_{plant}/1000\right)}$$ (Farquhar et al., 1989)

If $\delta^{13}C_{air}$ was –7.8‰ during the 1990s, and –6.5‰ in the Bronze Age (Indermuhle et al., 1999), then $\delta^{13}C_{einkorn}$ values of -22.4‰ and –23.5‰ for modern and Bronze Age wheat yield $\Delta_{einkorn}$ values of +14.9‰ and +17.4‰, respectively; i.e. a 2.5‰ higher value for the Bronze Age.

Of the various factors influencing Δ_{plant}, the availability of water exerts one of the strongest controls, and measurements of Δ_{plant} are widely used in assessing 'water use efficiency' (amount of carbon assimilated per unit of water transpired; Farquhar et al., 1989). Higher values for Δ_{plant} can reflect, for example, greater water availability or lower water use efficiency.

It must be acknowledged that the 2.5‰ higher value for $\Delta_{einkorn}$ for Assiros Toumba is based on samples which may only reflect the conditions of a single year. Our data nevertheless show marked similarities to historic changes reported for wheat in other parts of the Mediterranean. Thus Araus and Buxo (1993) reported Δ_{plant} = +15.5‰ and +17.1‰ for modern and Bronze Age durum wheat from Catalonia, and Araus et al. (2007) reported Δ_{plant} values of approximately +14‰ and +17‰ for modern and Neolithic durum wheat from the middle Euphrates. These changes can be interpreted both in terms of improvements in water use efficiency of modern varieties, or of cultivation under wetter conditions in the past (Araus and Buxo, 1993; Araus et al., 2007).

Wetter conditions for the wheat at Assiros might reflect a moister climate in the past, or growth in soils which are naturally wetter (alluvial terraces near rivers), or irrigation. Information on climate conditions in the eastern Mediterranean during the Bronze Age is equivocal with, as yet, no firm evidence for rainfall being substantially different from the present (Eastwood et al., 2007). The possibility that the Assiros cereals had access to additional water during the grain filling period, however, is consistent with earlier suggestions, on the basis of the crop weed flora, that cereals were cultivated under a garden regime (Jones, 1992), which probably entailed intensive tillage, and thus effective control of weeds that might otherwise have competed for available moisture, and may also have included artificial watering.

5. Conclusions

Within a single wheat field, changes in environmental conditions or plant physiology during the filling of the grains produce natural variations in the $\delta^{13}C$ values of individual grains of typically 0.5‰ (1 SD). The destructive nature of carbon isotope analysis means that, for precious archaeological samples, only a few grains may be available. Therefore, if the $\delta^{13}C$ values of samples from different archaeological contexts are to be regarded as significantly different based on analysis of, say five grains in each set, the mean value of each set must differ by at least 0.7‰. Samples of single species of wheat from modern harvests in Greece, however, have shown that harvests from different fields in the same region, or harvests from the same fields in different years, can sometimes have $\delta^{13}C$ values differing by less than 0.7‰. In these circumstances the spatial or temporal distinctions are not detectable. Larger, detectable differences between harvest locations or years probably require larger contrasts in climate.

The lack of significant differences between samples of einkorn from Bronze Age storerooms at Assiros Toumba is therefore consistent with a single year's harvest gathered over a small area, but the possibility that the grain represents harvests of different years, or from a wide area with little climatic variation, cannot be ruled out. Our study of modern crops near Assiros, however, suggests that whereas there is little difference in $\delta^{13}C$ values between locations within about 10 km, interannual variations over a ten year period are much larger. Thus, while it would be difficult to detect differences in $\delta^{13}C$ due to the pooling of crops from the local area, those resulting from the storage of crops grown over a period of several years should be detectable. We therefore conclude that while the $\delta^{13}C$ data are perfectly consistent with the pooling of local harvests in the Bronze Age storerooms, they are less likely to contain the harvest of several years or include grain imported from further afield. If the vast quantities of grain stored at this site do represent a single year's harvest, this suggests communal storage or mobilisation of grain by a ruling elite from the surrounding area – an interpretation consistent with archaeological indications of quite localised settlement hierarchies at this time in northern Greece (Andreou 2001).

The correlation of emmer and spelt $\delta^{13}C$ values provides strong evidence that these two wheat species were grown together as a maslin crop at Assiros, as has previously been argued on the basis of their co-occurrence in storage containers within the storeroom complex. It further suggests that the contents of different containers were derived from different fields and so, since individual containers probably held a few hundred litres of spelt/emmer maslin, opens the way to further tentative modelling of how the stored grain was mobilised. For example, if the stored produce represented the whole harvest, then individual fields are implied of a size that might be ploughed by a pair of cattle in a day (say 0.1-0.3 ha). Alternatively, if the containers held a small fraction (e.g. a tithe [tenth]) of the harvest, this implies fields large enough to exceed the daily ploughing capacity of a pair of powerful oxen. A weed flora compatible with intensive cultivation perhaps favours the former alternative.

^{13}C discrimination for einkorn grown in the Assiros region, $\Delta_{einkorn}$, appears to have decreased by about 2.5‰ between c. 1300 BC and modern times. It must be accepted that this is based on samples which may only represent a single year's harvest (i.e. might not be representative of 'average' conditions), and that a proper analysis of the significance of this change would require information on the extent to which modern varieties may have undergone genetic improvement in water use efficiency. In the absence of this, however, we can make comparison with studies of durum, where a 3‰

decrease in Δ_{durum} was interpreted to suggest that the 'total water inputs during grain filling' for Neolithic durum in the Middle Euphrates were 2-3 times higher than the rainfall inputs of the same area today (Araus et al., 2007). If a similar interpretation applies to Bronze Age Assiros, and points to the watering of the cereal crops or control of weeds that might compete for soil moisture, it would be consistent with the interpretation of the crop weed flora at Assiros, which indicates an intensive, horticultural regime of cereal cultivation.

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Table 1

Within-harvest variations in $\delta^{13}C$ values of modern wheat grain samples: bread wheat (cv. Mercia) from trial plots at Sutton Bonington (Nottingham, England); and durum wheat (cv. Athos) and einkorn from Greek agricultural research stations.

Location	Type of variation	Sample	Variation (s = sample standard deviation)
Sutton Bonington (‘Mercia’)	*Within spikelet* Average absolute difference between two grains from same spikelet	Ear 1, n = 5 grain pairs Ear 2, n = 5 grain pairs	average difference = 0.10‰ average difference = 0.13‰
Sutton Bonington (‘Mercia’)	*Within ear* Variation among single grains from different spikelet positions on same ear	Ear 1, n = 10 grains Ear 2, n = 8 grains	s = 0.41‰ s = 0.60‰
Sutton Bonington (‘Mercia’)	*Within plant* Variation among different ears on same plant (each ear sampled as composite of >50 grains)	Plant 1, n = 6 ears Plant 2, n = 6 ears Plant 3, n = 5 ears Plant 4, n = 6 ears	s = 0.47‰ s = 0.44‰ s = 0.83‰ s = 0.37‰
Sutton Bonington (‘Mercia’)	*Within plot* Variation among different plants in same plot (each plant sampled as composite of >50 grains)	870 m^2 plot, n = 6 plants	s = 0.29‰
Greece (Durum and einkorn)	*Within harvest* Variation among single grains from whole harvest of a trial plot. A = Agios Mamas; T = Thessaloniki. n = 6 grains in all cases.	Durum ‘Athos’, 1994, A Durum ‘Athos’, 1995, T Durum ‘Athos’, 2002, A Durum ‘Athos’, 2002, T Einkorn, 1993, A Einkorn, 1995, A	s = 0.61‰ s = 0.34‰ s = 0.32‰ s = 0.67‰ s = 0.59‰ s = 0.41‰

Table 2

$\delta^{13}C$ values of wheat grain (composite of 6 grains) from different locations and harvest years in Greece.

Site [a]	**Variety**	**Year**	**$\delta^{13}C$**
Assiros (ANLS)	Durum Simeto	1998	-24.7
Assiros (BFS)	Durum Simeto	1998	-24.4
Assiros (DT)	Durum Apulo	1995	-23.4
Assiros (EF)	Durum Apulo	1995	-22.6
Assiros (KLPTS)	Durum Simeto	1998	-24.7
Assiros (MG)	Durum Apulo	1995	-23.4
Assiros (PLKRNS)	Durum Simeto	1998	-24.3
Assiros (PTLTS)	Durum Cosmodur	1998	-24.1
Assiros (PNS)	Durum Cosmodur	1998	-23.6
Assiros (TL)	Durum Apulo	1995	-22.7
Assiros (TSGRS)	Durum Cosmodur	1998	-24.5
Assiros (TT)	Durum Apulo	1995	-22.8
Assiros (STS)	Durum Cosmodur	1998	-24.3
Assiros (TSTSS)	Durum Cosmodur	1998	-24.7
Agios Mamas	Durum Athos	1992	-24.8
Agios Mamas	Durum Athos	1994	-24.9
Agios Mamas	Durum Athos	1995	-23.8
Agios Mamas	Durum Athos	1996	-25.6
Agios Mamas	Durum Athos	1997	-23.9
Agios Mamas	Durum Athos	1998	-25.2
Agios Mamas	Durum Athos	1999	-25.6
Agios Mamas	Durum Athos	2000	-25.4
Agios Mamas	Durum Athos	2001	-23.3
Agios Mamas	Durum Athos	2002	-23.7
Agios Mamas	Einkorn	1991	-23.5
Agios Mamas	Einkorn	1992	-23.4
Agios Mamas	Einkorn	1993	-23.1
Agios Mamas	Einkorn	1994	-23.1
Agios Mamas	Einkorn	1995	-21.1
Agios Mamas	Einkorn	1996	-23.8
Agios Mamas	Einkorn	1998	-23.4
Examili	Durum Cosmodur	1998	-24.6
Haliartos	Durum Athos	1995	-25.5
Krithia	Durum Cosmodur	1998	-24.9
Krithia/Examili	Durum Cosmodur	1998	-24.2
Megali Volvi	Durum Cosmodur	1998	-25.5
Nea Zoe	Durum Athos	1994	-26.5
Nea Zoe	Durum Athos	1995	-25.5
Orestiada	Durum Athos	1995	-26.7
Serres	Durum Athos	1995	-26.2
Thessaloniki	Durum Athos	1993	-24.8
Thessaloniki	Durum Athos	1994	-26.1
Thessaloniki	Durum Athos	1995	-25.8
Thessaloniki	Durum Athos	1996	-26.1
Thessaloniki	Durum Athos	1997	-24.3
Thessaloniki	Durum Athos	1998	-24.7
Thessaloniki	Durum Athos	1999	-24.4
Thessaloniki	Durum Athos	2000	-25.7
Thessaloniki	Durum Athos	2001	-25.5
Thessaloniki	Durum Athos	2002	-25.6
Thessaloniki	Einkorn	1999	-23.5
Thessaloniki	Einkorn	2000	-23.1
Tripoli	Durum Athos	1995	-23.5

[a] Letters in parentheses refer to different farms around Assiros.

Table 3
Species differences in $\delta^{13}C$ values of grain (composite of 20-30 grains) of modern wheat samples from the John Innes Centre. Conditions: 1981 and 1985 outdoors in clay soil; 1992 under glass; 1999 outdoors in sandy soil

Sample	$\delta^{13}C$	%C	%N	C/N
Einkorn; D. 1981	-26.4	42.2	2.9	14.4
Emmer; D.1981	-25.4	41.4	2.7	15.4
Spelt; D. 1981	-24.3	42.3	3.3	13.0
Einkorn; D. 1985	-27.5	43.2	2.6	16.7
Emmer; D.1985	-26.1	42.6	2.7	15.6
Spelt; D. 1985	-24.7	41.5	2.8	14.8
Einkorn; S 55/92	-29.3	42.5	4.0	10.6
Emmer; S 55/92	-29.1	41.9	3.1	13.4
Spelt; D. S 55/92	-25.9	43.0	3.2	13.3
Einkorn; D. 1999	-26.5	42.9	2.6	16.6
Emmer; D.1999	-26.5	42.5	2.2	19.4
Spelt; D. 1999	-23.9	42.2	2.9	14.5

Table 4
Composition of charred wheat grains (n = 5 individual grains) from the Bronze Age site at Assiros Toumba, Greece. Data are averages of 5 individual grains (with sample standard deviations in parentheses), or single analysis of composite of 5 grains.

Sample	δ^{13}C	%C	%N	C/N
Einkorn				
739 EK	-22.96 (0.32)	46.9 (1.30)	3.5 (0.70)	13.7 (2.34)
906 EK	-23.92 (0.43)	47.9 (1.74)	2.5 (0.10)	19.2 (0.95)
927 EK	-23.11 (0.36)	47.3 (1.26)	4.3 (0.26)	11.0 (0.89)
965 EK	-23.29 (0.37)	48.0 (2.40)	2.7 (0.34)	17.9 (1.65)
1000 EK	-23.45 (0.67)	47.6 (3.04)	2.6 (0.10)	18.1 (0.67)
1007 EK	-24.19 (0.51)	40.7 (1.81)	2.3 (0.23)	17.8 (1.32)
1026 EK	-23.45 (0.45)	51.3 (10.31)	3.9 (1.76)	14.5 (3.61)
1115 EK	-23.49 (0.55)	51.3 (2.57)	3.1 (0.34)	16.8 (1.14)
2292 EK	-23.52 (0.36)	47.6 (1.28)	3.1 (0.14)	15.2 (0.89)
3202 EK	-23.77 (0.41)	51.5 (0.68)	3.1 (0.20)	16.6 (0.93)
2427 EK	-23.96 (0.38)	59.7 (9.33)	3.4 (0.43)	17.7 (1.38)
2455 EK	-23.44 (0.40)	53.1 (4.26)	2.7 (0.60)	20.1 (3.51)
2459 EK	-23.42 (0.40)	51.3 (1.84)	3.1 (0.35)	16.6 (2.27)
2469 EK	-23.48 (0.37)	52.0 (9.10)	3.5 (0.90)	15.2 (3.00)
2496 EK	-23.32 (0.40)	50.3 (5.24)	3.4 (0.69)	15.2 (2.13)
2506 EK	-23.59 (0.41)	47.8 (2.57)	2.5 (0.20)	19.6 (2.34)
2540 EK	-23.74 (0.44)	57.5 (5.09)	2.5 (0.33)	22.8 (1.96)
2545 EK	-23.23 (0.42)	47.6 (2.49)	2.6 (0.31)	18.8 (2.34)
2565 EK	-23.76 (0.26)	52.6 (1.34)	3.4 (0.18)	15.6 (1.01)
2593 EK	-23.48 (0.17)	37.3 (3.57)	2.0 (0.28)	18.9 (1.26)
2637 EK	-23.56 (0.57)	48.1 (2.14)	2.9 (0.48)	16.9 (2.79)
2648 EK	-24.06 (0.34)	58.5 (4.95)	3.4 (0.29)	17.2 (2.12)
3529 EK	-23.58 (0.53)	48.1 (5.19)	2.9 (0.43)	16.7 (1.90)
4396 EK	-23.20 (0.57)	43.9 (2.43)	2.6 (0.44)	17.5 (2.07)
Emmer				?
630 EM	-23.51 (0.61)	46.8 (4.79)	2.6 (0.48)	17.9 (2.41)
735 EM	-22.73 (0.16)	52.6 (1.03)	3.5 (0.22)	15.3 (0.84)
907 EM	-22.45 (0.40)	51.2 (2.01)	3.1 (0.53)	16.7 (3.00)
1021 EM	-23.00 (0.27)	49.6 (1.61)	2.6 (0.13)	19.3 (1.77)
1136 EM	-23.01 (0.79)	48.1 (2.76)	2.7 (0.35)	18.0 (2.40)
2308 EM	-23.08	47.7	2.7	17.6
2591 EM	-23.08	57.5	3.1	18.6
2691 EM	-22.53	47.2	2.7	17.3
2700 EM	-23.09	46.6	2.7	17.0
3219 EM	-23.26	49.4	2.9	17.2
4224 EM	-22.69	48.5	2.9	16.5
4656 EM	-23.12	47.3	2.9	16.6
5035 EM	-22.78	50.8	3.0	17.1
5080 EM	-22.81	49.1	2.8	17.3
5120 EM	-22.82	45.8	2.5	18.4
5126 EM	-22.38	40.0	2.2	18.1
Spelt				?
630 SP	-23.21 (0.55)	47.1 (5.08)	2.8 (0.42)	17.0 (2.02)

735 SP	-23.00 (0.41)	49.8 (1.71)	4.1 (0.53)	12.1 (2.57)
907 SP	-22.95 (0.85)	51.4 (2.25)	3.3 (0.26)	15.9 (1.40)
1021 SP	-22.90 (0.52)	50.4 (1.01)	3.2 (0.33)	15.8 (1.81)
1136 SP	-23.11 (0.56)	49.9 (0.76)	3.0 (0.31)	16.7 (2.17)
2591 SP	-23.37	61.4	3.5	17.4
2691 SP	-22.31	47.9	3.0	16.1
3219 SP	-23.10	50.2	3.0	16.8
4206 SP	-23.31	47.7	2.7	17.6
5080 SP	-23.03	50.4	2.6	18.8
5120 SP	-22.71	44.7	2.7	16.5

Figure Captions

Fig. 1. Map of Greece showing sample locations.

Fig. 2. Plan of the Assiros storeroom complex showing locations of grain samples (cf Wardle (1989) fig. 4). m=einkorn (*Triticum monococcum*); d=emmer (*T. dicoccum*); s=spelt (*T. spelta*).

Fig. 3. $\delta^{13}C$ values of the grain (solid symbols) and chaff (open symbols) from different spikelet positions, from the base to the terminus, on ears of two wheat plants ('Mercia' bread wheat, Sutton Bonington).

Fig. 4. $\delta^{13}C$ values of einkorn (open circles) and durum wheat (crosses) from trial plots at Agios Mamas (solid lines) and Thessaloniki (broken line), grown in different years. From data in Table 2.

Fig. 5. $\delta^{13}C$ values of different varieties of durum wheat grown in different parts of Greece in different years. From data in Table 2.

Fig. 6. Effect of charring on the $\delta^{13}C$ values and C/N ratios of einkorn grains from: (a) Agios Mamas, Greece; (b) southern France. Heating was for 3 days at 210, 220, 240 and 250°C, or for 5 days at 210, 220 and 240°C. Grains were either open to the atmosphere, or closed to the atmosphere by wrapping in foil and burying in sand. Large open circle = composition of original uncharred grains. Individual treatments were not replicated.

Fig. 7. Histogram distributions of the $\delta^{13}C$ values of charred Bronze Age einkorn, emmer and spelt from Assiros Toumba. The value for each sample is the average of five grains.

Fig. 8. Co-variation in the $\delta^{13}C$ values of paired emmer and spelt samples from storage containers at Assiros Toumba, from data in Table 4.The value for each sample is the average of five grains. Numbers along abscissa are sample numbers (see Fig. 2) arranged in order of increasing average emmer/spelt $\delta^{13}C$ values.

A = Assiros**; AM** = Agios Mamas; **Ex** = Examili; **K** = Krithia; **MV** = Megali Volvi; **NZ** = Nea Zoe; **T** = Thessaloniki

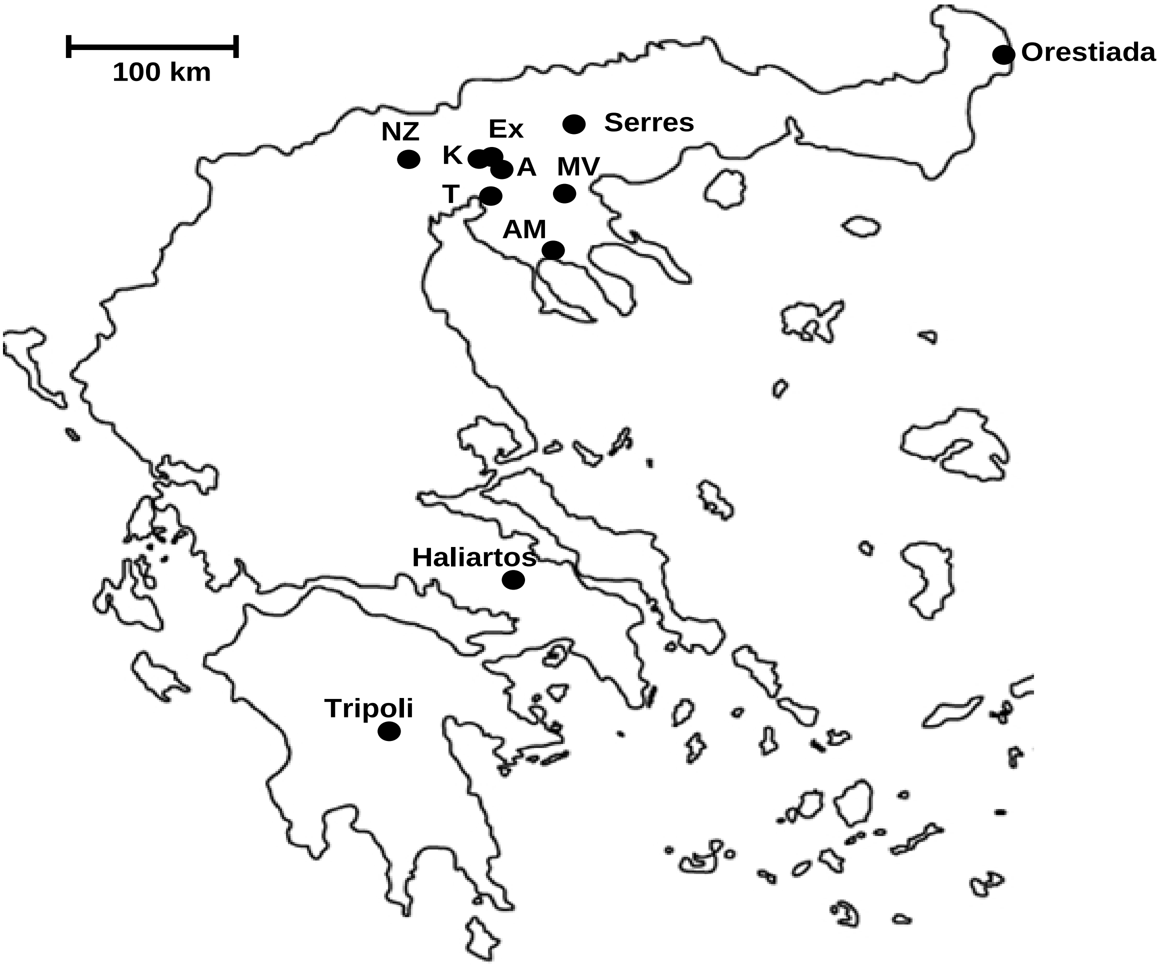
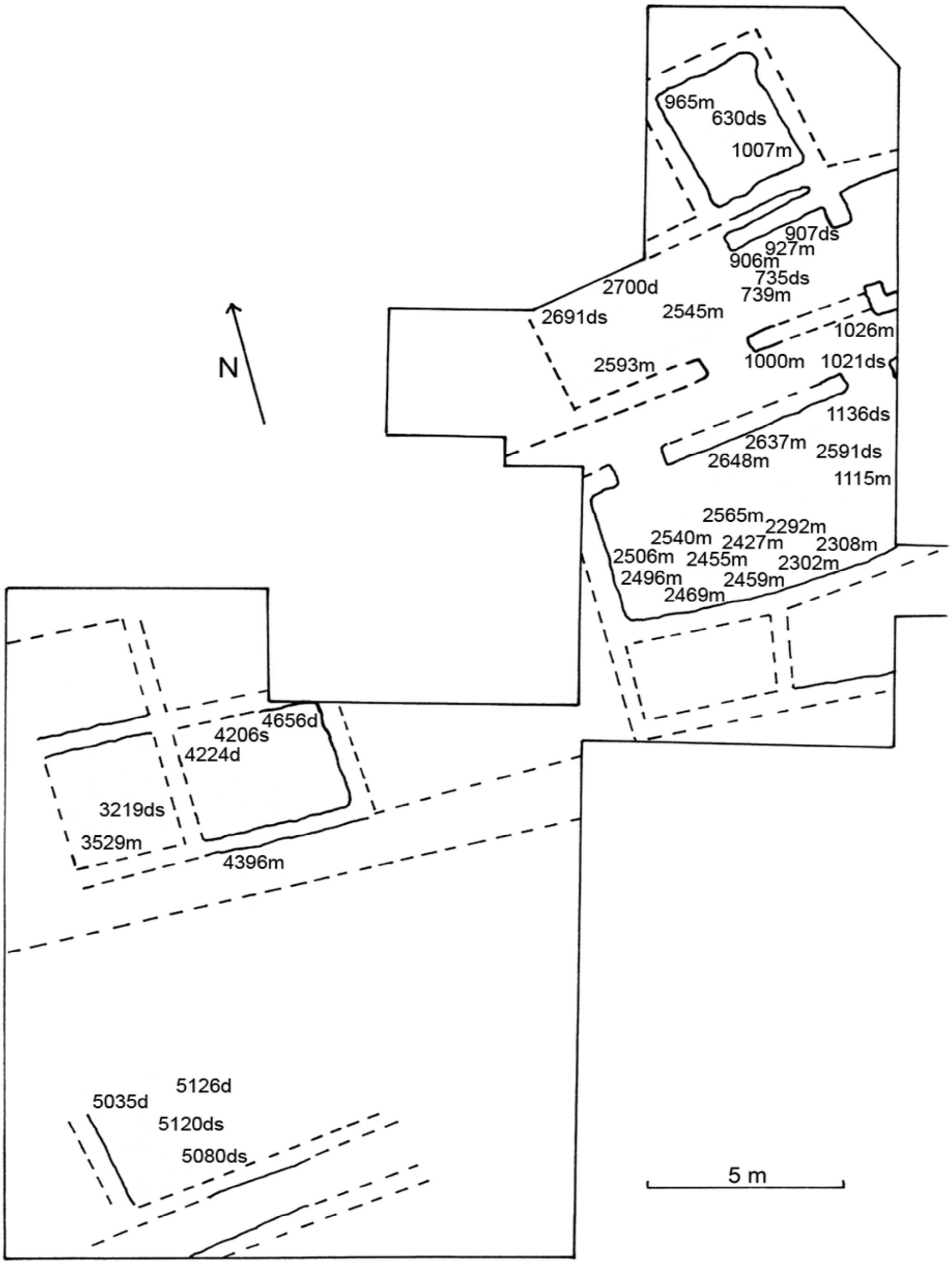


FIGURE 1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2459m
2469m
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3529m
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5126d
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5 m

FIGURE 2

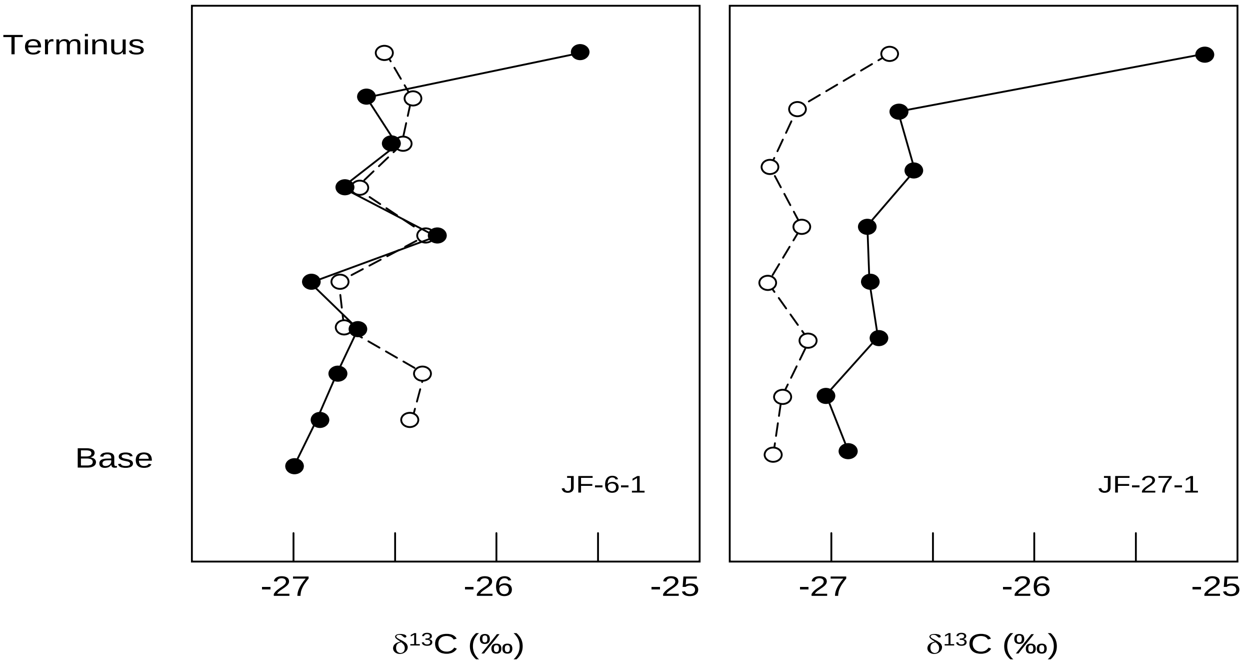


FIGURE 3

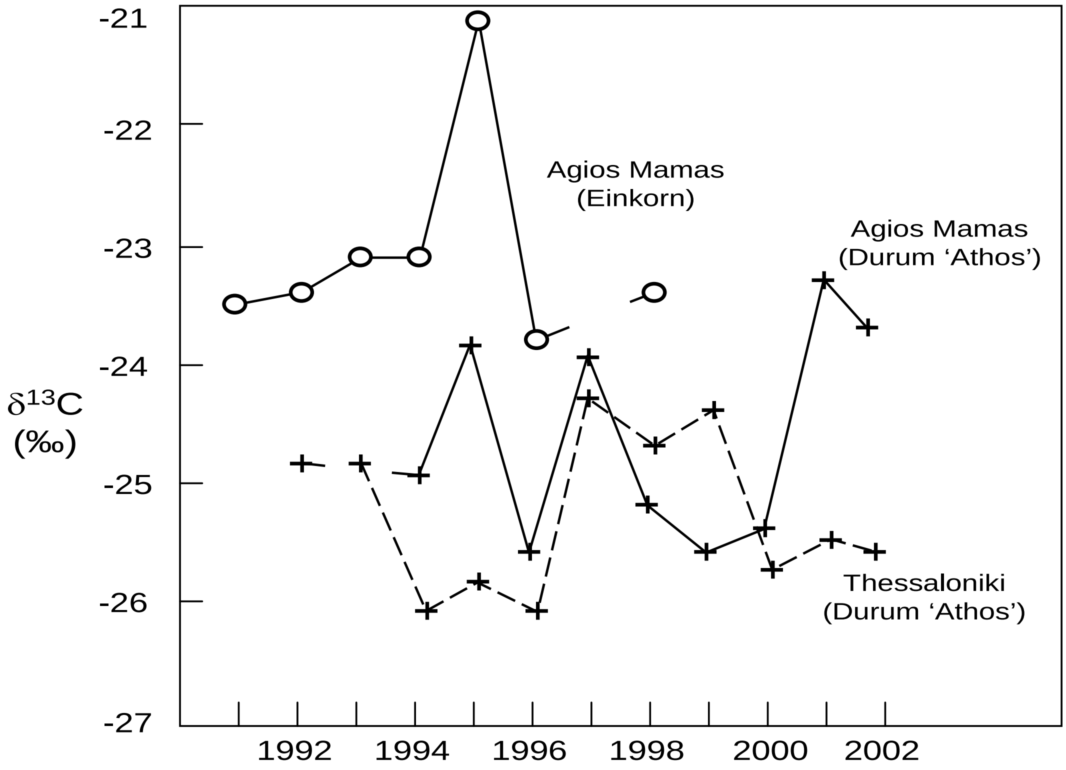

-21
-22
-23
-24
-25
-26
-27
$\delta^{13}C$ (‰)
1992
1994
1996
1998
2000
2002
Agios Mamas (Einkorn)
Agios Mamas (Durum 'Athos')
Thessaloniki (Durum 'Athos')

FIGURE 4

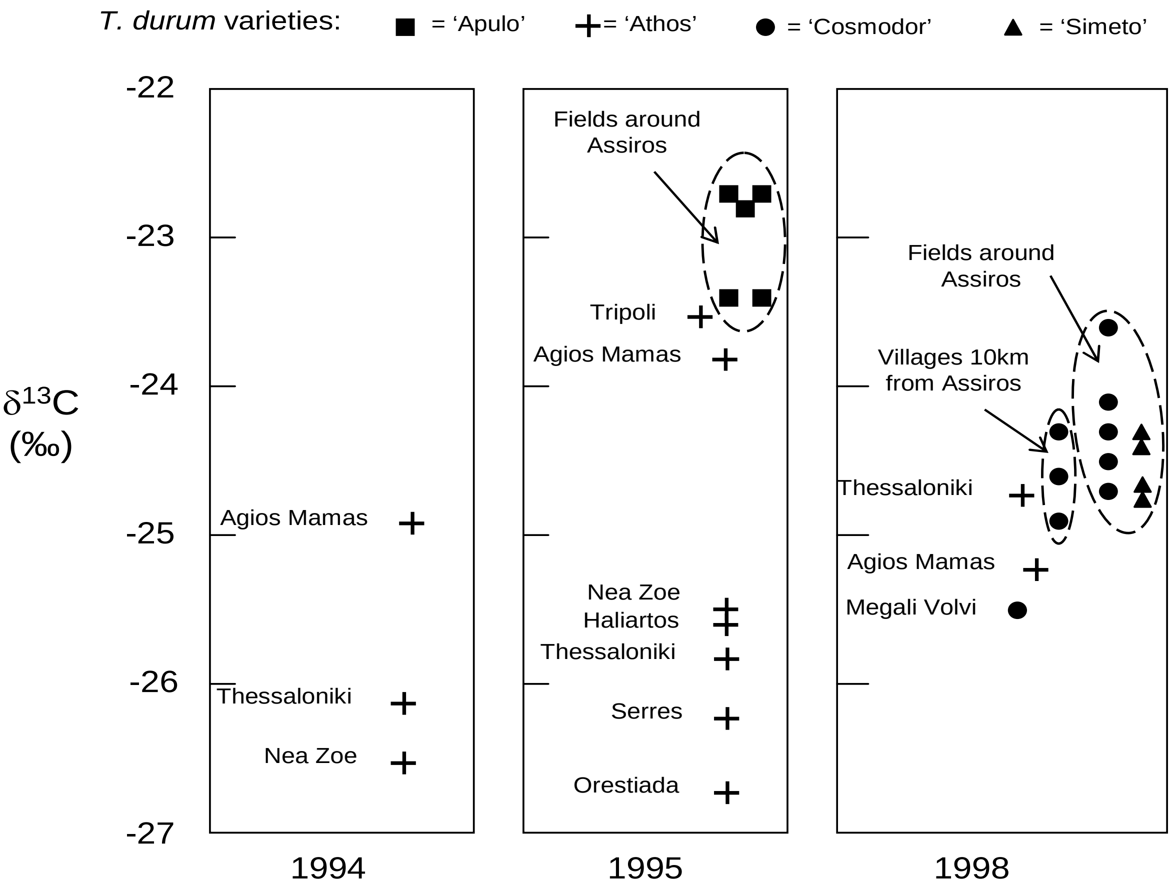

T. durum varieties:
■ = 'Apulo'
+ = 'Athos'
● = 'Cosmodor'
▲ = 'Simeto'
$\delta^{13}C$ (‰)
-22
-23
-24
-25
-26
-27
Agios Mamas
Thessaloniki
Nea Zoe
1994
Fields around Assiros
Tripoli
Agios Mamas
Nea Zoe
Haliartos
Thessaloniki
Serres
Orestiada
1995
Fields around Assiros
Villages 10km from Assiros
Thessaloniki
Agios Mamas
Megali Volvi
1998

FIGURE 5

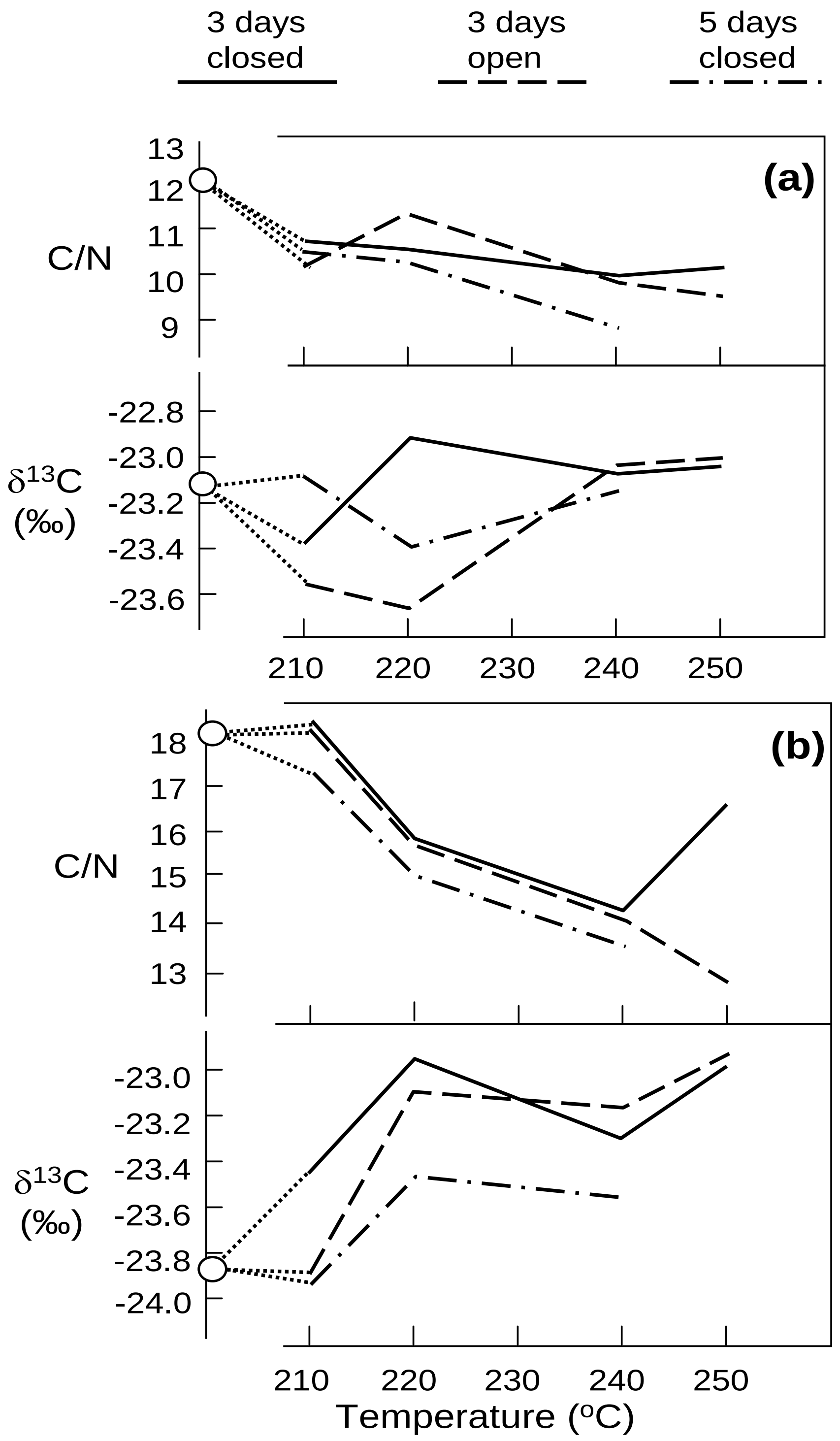
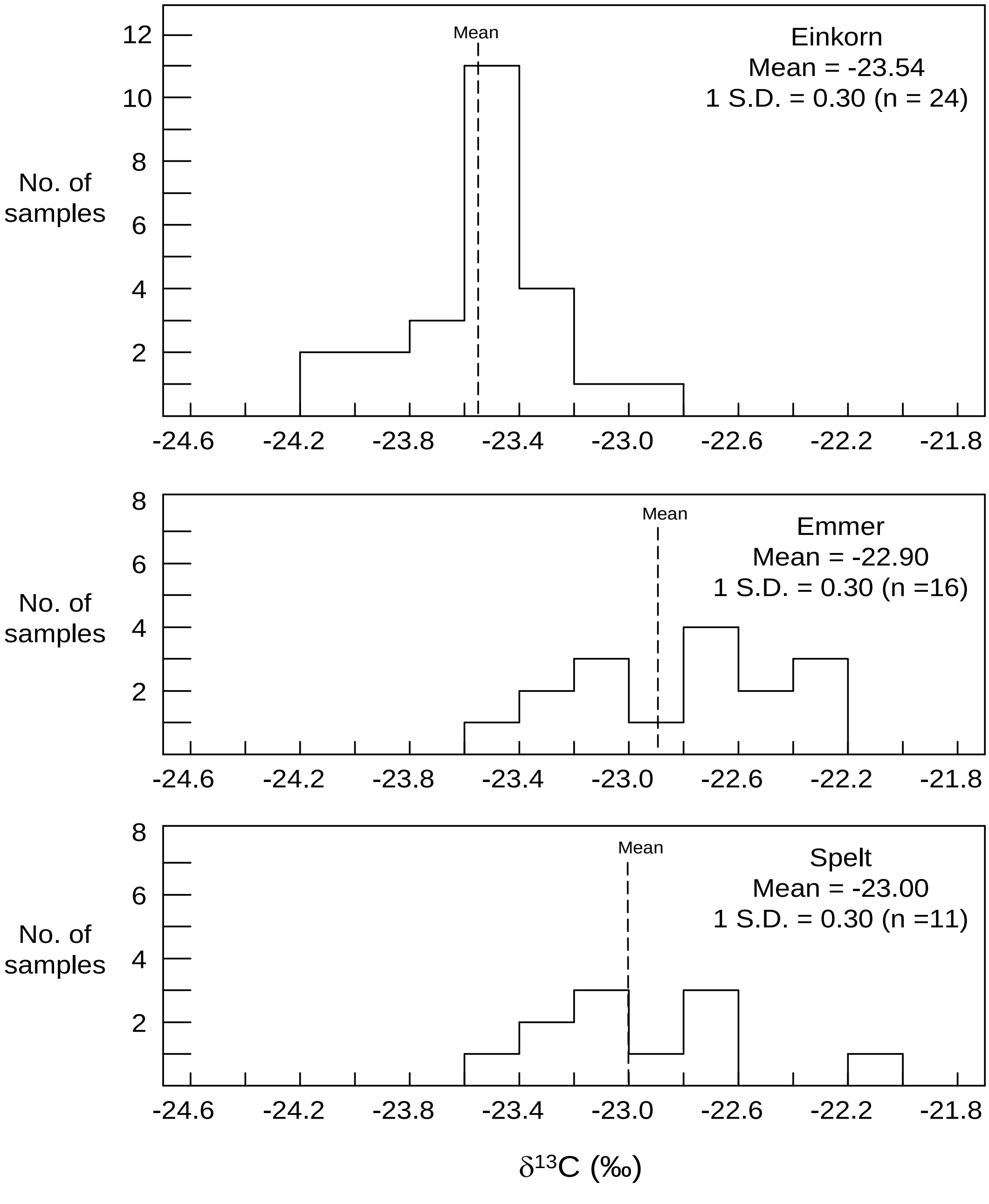

3 days closed
3 days open
5 days closed
(a)
C/N
13
12
11
10
9
δ13C (‰)
-22.8
-23.0
-23.2
-23.4
-23.6
210
220
230
240
250
(b)
C/N
18
17
16
15
14
13
δ13C (‰)
-23.0
-23.2
-23.4
-23.6
-23.8
-24.0
210
220
230
240
250
Temperature (oC)

FIGURE 6



Einkorn
Mean = -23.54
1 S.D. = 0.30 (n = 24)
Mean
No. of samples
12
10
8
6
4
2
-24.6
-24.2
-23.8
-23.4
-23.0
-22.6
-22.2
-21.8
Emmer
Mean = -22.90
1 S.D. = 0.30 (n =16)
Mean
No. of samples
8
6
4
2
Spelt
Mean = -23.00
1 S.D. = 0.30 (n =11)
Mean
No. of samples
8
6
4
2
δ13C (‰)

FIGURE 7

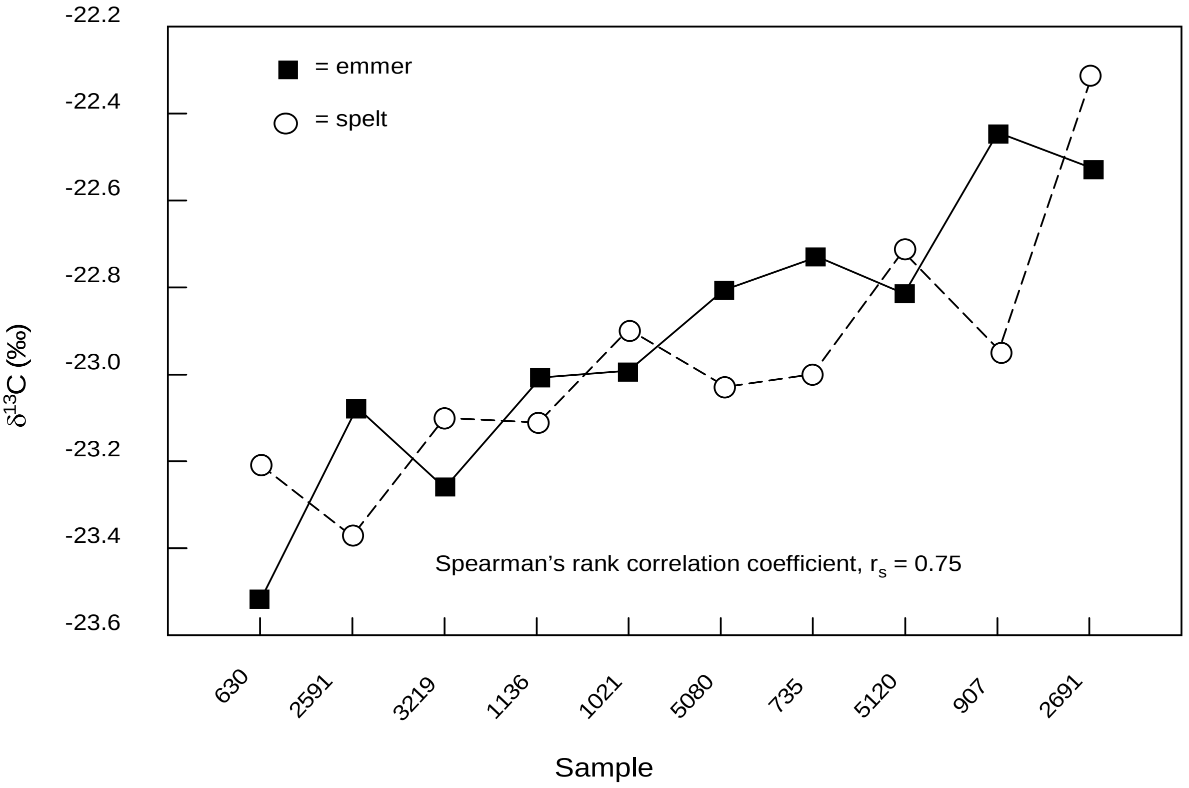

-22.2
-22.4
-22.6
-22.8
-23.0
-23.2
-23.4
-23.6
δ13C (‰)
■ = emmer
○ = spelt
Spearman's rank correlation coefficient, r_s = 0.75
630
2591
3219
1136
1021
5080
735
5120
907
2691
Sample

FIGURE 8