Title: An integrated stable isotope study of plants and animals from Kouphovouno, southern Greece: a new look at Neolithic farming

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Abstract:

This paper presents the first study that combines the use of ancient crop and animal stable isotopes (carbon and nitrogen) and Zooarchaeology Mass Spectrometry species identification (ZooMS) for reconstructing early farming practices at Kouphovouno, a Middle-Late Neolithic village in southern Greece (c. 5950-4500 cal. BC). Debate surrounding the nature of early farming predominantly revolves around the intensity of crop cultivation; did early farmers move around the landscape while practicing temporary farming methods such as slash and burn agriculture or did they create more permanent fields by investing high labor inputs into smaller pieces of land that produced higher crop yields? The need to address these questions using a direct assessment of the intensity and scale of cultivation is apparent, and an integrated stable isotope approach provides such an opportunity. The results of this study support the model of small-scale mixed farming, where crop cultivation and animal husbandry are closely integrated. The farmers directed their intensive management towards crops grown for human consumption (free-threshing wheat), while growing fodder crop (hulled barley) more extensively. Pulses were cultivated under a high-manuring/high-watering regime, likely in garden plots in rotation with free-threshing wheat. The diets of the livestock enable us to investigate which parts of the landscape were used for browsing and grazing and indicate that animal management changed in the Late Neolithic. The sheep and goats were now kept in smaller numbers and grazed together and new pasture grasses were sought for the grazing of cattle. This study demonstrates that beyond its applicability for palaeodietary reconstruction, analysis of stable isotopes of archaeological crop and animal remains has important implications for understanding the relationship between humans, plants and animals in an archaeological context.

Keywords: stable isotopes, carbon, nitrogen, ZooMS, archaeobotany, archaeozoology, Aegean, Neolithic

1. Introduction

Recent investigations in the field of stable isotope analysis have demonstrated the potential of obtaining *direct* evidence of ancient agricultural practices from $\delta^{15}N$ and $\delta^{13}C$ analysis of archaeobotanical remains (Araus et al. 1997, 1999; Bogaard et al. 2007, 2013; Fraser et al. 2011, 2013a; Kanstrup et al. 2011, 2012, 2013; Lightfoot and Stevens 2012; Wallace et al. 2013). Analysis of modern crops grown under a range of experimental and traditional farming regimes has shown that $\delta^{15}N$ and $\delta^{13}C$ values reflect crop growing conditions, such as soil nitrogen composition and crop water availability. In addition, some of this work (Bogaard et al. 2007; 2013; Fraser et al. 2011, 2013a, 2013b; Lightfoot and Stevens 2012) has highlighted the fact that where possible, the isotopes of plants must be measured alongside the isotopes of human and faunal remains in order to better understand ancient palaeodiets.

The current paper builds on this work by integrating the analysis of ancient crop and faunal remains in order to explore the nature of agricultural economy at the Middle-Late Neolithic site of Kouphovouno (5950-4500 cal. BC). It has long been argued that management of domestic plants and animals in a farming context can be functionally interdependent (Bogaard 2004a, 2004b, 2005; Bogaard and Isaakidou 2010; Byrd 2000; Flannery 1969; Halstead 1989). Animals can be used to regulate and promote crop productivity as well as for "indirect storage" of surplus grain and crops/arable fields can serve as fodder and graze for the animals. In light of this view, it has been suggested that early farming systems in Greece, and in fact the rest of Europe, employed some version of a small-scale mixed farming economy (Bogaard 2004a, 2004b, 2005; Halstead 1996, 2000, 2006). Thus, the aim of this paper is to investigate if the stable isotope data support or refute an intensive mixed farming model and if the changes in

pottery styles observed between the Middle and Late Neolithic accompanied by shifts in agricultural management.

2. Methodological background

Stable isotope signatures of archaeological material (such as bone collagen or charred plant material) are useful for reconstructing not only past human and animal diets, but practices such as land use and agricultural management (for examples of palaeodietary studies, see Chisholm et al. 1982; Richards et al. 2003; Schulting and Richards 2002; Schwarcz et al. 1985). The stable isotope values of carbon and nitrogen, expressed as δ^{13} C and δ^{15} N, represent the ratios of the heavier to lighter isotopes (13 C: 12 C and 15 N: 14 N) in a given sample compared to internationally set standards (PeeDee Belemnite for carbon and AIR for nitrogen). The ratios are expressed in parts per thousand, ‰, and are calculated using the following equation:

For a comprehensive review of the technique, see Lee-Thorp (2008), O'Connell and Hedges (1999), Schoeninger and Moore (1992), Schwarcz and Schoeninger (1991).

Plants and animals obtain carbon and nitrogen through metabolic processes such as breathing, photosynthesis and digestion. These processes are governed by principles of biochemical fractionation that determine the proportions in which the different isotopes of each element get assimilated into the organism's tissues (such as animal muscles/bones, and crop grain/straw). When another organism consumes those tissues, the isotope chemistry of the consumer reflects the distinct signature of the food source plus an enrichment factor. Thus, herbivores have higher δ^{15} N and to a lesser extent δ^{13} C

compared to the plant foods they consume and carnivores higher values than the herbivores they eat (Bocherens et al. 1995; Chisholm et al. 1982; DeNiro and Epstein 1978; Hedges 2006; Hedges and Reynard 2007; Lee-Thorp et al. 1989; Sillen et al. 1989; Vogel and van der Merwe 1977). Collagen δ^{13} C and δ^{15} N values predominantly reflect the protein portion of the consumers' diets (Ambrose and Norr 1993; Chisholm et al. 1982), with many amino acids being directly assimilated from the food source (Chikaraishi et al. 2009; Corr et al. 2005; McCullagh et al. 2006).

The δ^{15} N of crops reflects the isotopic composition of their nitrogen source. N₂-fixers, such as lentils and other pulses, host *Rhizobium* bacteria in the nodules of their roots, which convert atmospheric nitrogen to ammonium ions and pass them to the plant or surrounding soil. This process does not cause substantial fractionation and as a result, these plants have δ^{15} N values closer to the value of atmospheric nitrogen, which is 0‰ (Bernhard 2012; Delwiche et al. 1979; Högberg 1997). Non-N₂-fixers, such as cereals, obtain their nitrogen from the soil and are thus directly affected by the factors that cause soil ¹⁵N enrichment discussed below (Bogaard et al. 2007; Fraser et al. 2011; Kanstrup et al. 2011, 2013). Although in traditional farming contexts, N₂-fixers fix mostly atmospheric nitrogen, they are also affected by manuring, albeit to a lesser extent than non-N₂-fixers (Fraser et al. 2011). A possible mechanism for this is direct uptake of amino acids present in soil (as has been found to be the case in the non-leguminous wheat (Hill et al. 2011)).

Soil ¹⁵N enrichment can be caused by natural factors such as soil salinity (Ambrose 1991; Heaton 1987; although for opposite argument see Yousfi et al. 2010), sea-spray of nitrates (Heaton 1987; Virginia and Delwiche 1982), and denitrification (Högberg 1997; Tiedje et al. 1982). Significant denitrification occurs in waterlogged conditions such as

marshlands. Elevated δ^{15} N values have also been correlated with decreased precipitation in experimental and field studies in arid regions in South Africa, the southwestern United States, Namibia, and the Kenya Rift Valley (Ambrose 1991; Evans and Ehleringer 1993, 1994; Heaton 1987; Heaton et al. 1986; Lopes and Araus 2006; Sealy et al. 1987; Swap et al. 2004). Aranibar et al. (2004) observed an aridity effect across a precipitation gradient (978 – 230mm of annual rainfall) in the Kalahari desert, but argue that ¹⁵N enrichment in arid climates may be more affected by unpredictability of precipitation rather than lower overall annual rainfall levels. Fraser et al. (2011) assert that the aridity effect may be buffered through crop management and therefore invisible in agricultural settings.

Anthropogenic factors that cause ¹⁵N enrichment include middenning (cf Guttmann 2005; Bogaard 2012), manuring (Bogaard et al. 2007; Fraser et al. 2011; Kanstrup et al. 2011, 2013), clear-cutting of forests (Pardo et al. 2002) and burning of fossil fuels (Templer et al. 2007).

 δ^{15} N from animal and human collagen can be used to assess the *trophic* level of the individual, which is an indication of where the individual was situated in the food chain (DeNiro and Epstein 1981; Hedges and Reynard 2007; Minagawa and Wada 1984). For many years, the widely accepted value for isotopic spacing between diet and consumer tissue has been 3-5‰ (Ambrose 2000; Bocherens and Drucker 2003; Hedges and Reynard 2007), but recent work on isotope spacing in humans by O'Connell et al. (2012) suggests that this value may be higher, between 4.6-6‰. Taking all of this work into account and assuming that trophic enrichment for humans is comparable to that for domestic animals, in this study, we use 5 ± 1‰ as an approximation of tissue-diet spacing.

Plant δ^{13} C values are determined by the δ^{13} C of the atmospheric CO₂ at the time of the plant's growth as well as the degree of fractionation that occurs during photosynthesis. Since the concentration of CO₂ in the atmosphere has fluctuated over time, differences in plant δ^{13} C values from different periods do not simply reflect growing conditions and fractionation, but the isotopic composition of the carbon source as well. For this reason, when comparing crop δ^{13} C across different periods, the values need to be converted into Δ^{13} C, which reflect the carbon isotope discrimination independent of atmospheric CO₂ concentration (Farquhar et al. 1982; 1989; Jackson et al. 1994; Wallace et al. 2013). The conversion is done following the principles outlined by Farquhar et al. (1989) and using the equation:

 $\delta^{13}C_{air}$ is the isotopic composition of atmospheric CO₂ at the time that the plant was growing. These values have been preserved in sequence in Antarctic ice-cores and can be estimated using the AIRCO2_LOESS data calibrator¹ for the time period between 16,100 B.C.E. and 2003 C.E. (Cleveland et al. 1979; Ferrio et al. 2005; Francey et al. 1999; Indermühle et al. 1999; Lauenberger et al. 1992). Converting $\delta^{13}C$ into $\Delta^{13}C$ changes the directionality of the effect (i.e. higher $\delta^{13}C$ values equate to lower $\Delta^{13}C$ values), and the sign (negative $\delta^{13}C$ values become positive $\Delta^{13}C$ values).

Carbon isotope discrimination in plants, encoded in their Δ^{13} C, is driven by the photosynthetic mechanism. Many factors are involved in this process (including light intensity, temperature, and concentration of CO₂ in the atmosphere), but it is argued that

¹ http://web.udl.es/usuaris/x3845331/AIRCO2_LOESS.xls

in semi-arid climates, Δ^{13} C is most notably affected by water availability (Farquhar et al. 1982, 1989; Farquhar and Richards 1984; Wallace et al. 2013). The amount of water available controls the action of the plant stomata (pores on the surface of the plants), which affects how the CO₂ is used during photosynthesis. When sufficient amounts of water are available, the plant stomata stay open for longer periods of time and CO₂ is replenished during photosynthesis so that more of the lighter ¹²C (which reacts more quickly in chemical reactions) is available for assimilation. When the water supply is limited, the plant periodically closes its stomata to conserve the water. This causes the CO₂ to be recycled and more of the heavier ¹³C to be assimilated (Farquhar et al. 1982, 1989; O'Leary et al. 1981; Smith et al. 1976; Wallace et al. 2013). Measurement of Δ^{13} C of crops is thus instrumental for inferring the water status of the crops during their growth season.

3. The site

Kouphovouno is located in mainland southern Greece, c. 2.5km southwest of Sparta on the Peloponnesian peninsula (see Fig. 1). The was first occupied in the Middle Neolithic, which, in Southern Greece is dated to c.5950 - 5450 cal BC, and continued through the Late Neolithic (c. 5450 - 4500 cal BC). Based on AMS carbon dating of seeds from the same contexts, the strata represented in this study date to a narrower range of c. 5800 -5000 cal. BC (Mee et al. in press).

First excavation of the site was undertaken by O-W. von Vacano in 1941 and this work was published by Josette Renard in 1989. The initially exposed strata indicated that the site had been occupied between the Middle Neolithic and the Early Helladic II periods. The site was reopened in 2001 after two seasons of survey, which indicated that a number of burnt structures lay buried under the surface and that the extent of occupation

of the site did not vary significantly between the Middle and the Late Neolithic (Cavanagh et al. 2004, 2007; Mee et al. in press.).

A further five seasons of excavation recovered well-preserved botanical and faunal assemblages across the excavated Areas A-G in contexts ranging from burnt floors and hearth structures to destruction layers and midden deposits. The available archaeology indicates that the settlement was nucleated and may have been divided into neighborhoods. The plant assemblage mostly consists of domestic species of cereals and pulses including free-threshing wheat, hulled barley, (one-seeded) einkorn, emmer, lentil, common pea, grass pea, and bitter vetch; along with figs and flax. The faunal assemblage is dominated by domestic animals such as cattle, sheep, goats, pigs and dogs, but wild animals such as red deer, roe deer, wild boar, aurochs, wild goat, hare, wolf, fox, cat and weasel were also found in smaller quantities (Cantuel et al. 2008; Cavanagh et al. 2004; Gardeisen 2007).

The surrounding Sparta Basin is one of the most fertile regions in Greece, and ample arable land was available for cultivation in the Neolithic. The early farmers may have sown their crops in the floodplain on which the tell sits, on alluvial fans located 300m to the west of the site and/or on unoccupied parts of the tell itself. The environment also offered land suitable for animal grazing/browsing and plentiful drainage from nearby rivers (Fouache et al. 2007; section by James and Kousoulakou in Cavanagh et al. 2004).

4. Materials and Methods

4.1. Choice of samples

28 bulk plant samples of charred archaeobotanical material were analyzed in this study. The samples include 13 samples of free-threshing wheat grain (*Triticum aestivum* L./*Triticum durum* Desf.), 7 samples of hulled barley grain (*Hordeum vulgare* L.), 7 samples of common pea (*Pisum sativum* L.) and 1 sample of lentil (*Lens culinaris* Medik.). Other species recovered in the archaeobotanical record (Bogaard unpublished data) include wheat of the hulled variety: einkorn (*Triticum monococcum* L.) and emmer (*Triticum dicoccum* Shrank); these were recovered in smaller quantities, however, and could not be included in the isotope sampling

68 samples of animal bone were analyzed in this study. These included 15 samples of domestic cattle (*Bos taurus*), 7 dogs (*Canis lupus familiaris*), 12 domestic sheep (*Ovis aries*), 7 domestic goats (*Capra hircus*), 23 domestic pigs (*Sus scrofa domesticus*), 1 wild boar (*Sus scrofa scrofa*), 1 hare (*Lepus europaeus*), 1 bear (*Ursus arctos*) and 1 sample of wild goat (*Capra aegagrus*/*Capra ibex*).

See Supplementary Table 1 for details on the archaeological context of the samples. Due to the limited number of samples, we have grouped the samples only into two chronological phases: Middle Neolithic and Late Neolithic. Elsewhere, some of these contexts are discussed to be Transitional MN/LN (Mee et al. in prep). In this study, the transitional contexts are treated as Late Neolithic due to the occurrence of Black Ware pottery, which is taken to signal the onset of Late Neolithic.

Insert Supplementary Table 1 here.

4.2. Wild vs. domestic plants

Most of the samples analyzed in this study represent domestic forms of plants and animals. While it would be useful to have a large sample size of wild animals for comparison, the 1 sample of bear, 1 sample of hare, 1 sample of wild goat and 1 sample of wild boar were all that was available from the wild faunal assemblage. As for the crops, there are two reasons why we did not sample any wild species. First, the only wild plant that is available in a usable quantity from this site is fig and measuring it would not provide us with any useful information. It could not serve as a reference for the "environmental background" as it is unknown where the fig trees grew. They may have been located adjacent to the fields and thus been affected by arable management. Additionally, the isotopic composition of this one species would not illustrate the diversity of the isotopic composition of all the grasses and browse available for the herbivores to consume. Second, we hesitate to measure the stable isotopes of wild plant species, as these have not been studied experimentally and so it is unknown how these plants respond to charring and soil ¹⁵N enrichment. In light of these limitations, the interpretations made in this study are primarily based relative differences between (predominantly domestic) species, and reference is made to forage values inferred from local herbivore collagen values by subtracting a trophic enrichment factor.

4.3. Pre-treatment

Pre-treatment of the plant material followed the acid-base-acid protocol described by Fraser et al. (2011). The samples were first soaked in 0.5M HCl at 70°C for 30-60min and subsequently rinsed 3 times in distilled water. Next, they were soaked in of 0.1M NaOH at 70°C for 60min and rinsed as many times as it took to wash out the brown humic substances (up to 9 rinses). Lastly, the samples were heated in 0.5M HCl at 70°C for 25min and rinsed 3 times in distilled water. Kanstrup et al. (2013) used stronger acid and base to pre-treat their archaeobotanical samples (1M HCl and 1M NaOH for 1h, 3h

and 16h). They found a difference of 0.9‰ between their pre-treated and non-pretreated samples; however, it remains to be determined whether this difference was solely due to the removal of contamination or the use of more vigorous pre-treatment.

Pre-treatment of the faunal material followed the standard procedure for bone collagen extraction (modified Longin (1971) method described by Richards and Hedges (1999)). Bone pieces were demineralized using 0.5M HCl at 5°C over a period of 3-10 days and subsequently washed three times in distilled water. The residue was gelatinized in pH3 HCl solution for 48h at 75°C and subsequently freez e-dried.

4.4. Isotope measurements and precision

Measurements of plant δ^{13} C were performed at the NERC Isotope Geosciences Laboratory on a Costech 4010 on-line to a VG TripleTrap and Optima dual-inlet mass spectrometer. Measurements of plant δ^{15} N and faunal δ^{13} C and δ^{15} N were analyzed using Continuous Flow Isotope Ratio Mass Spectrometry (CFIRMS) comprised of an Elemental analyser (Flash/EA) coupled to a Thermo Finnigan Delta^{Plus} XL isotope ratio mass spectrometer via a ConFlo III interface. Collagen δ^{13} C and δ^{15} N ratios were calibrated using internal reference material M1360p (powdered gelatine from British Drug Houses) with expected δ^{13} C values of –20.32‰ (calibrated against IAEA CH7) and +8.12‰ (calibrated against IAEA N-1 and N-2) for C and N respectively. Plant δ^{13} C and δ^{15} N ratios were calibrated using internal reference material BROC-2 (powdered broccoli) with expected delta values of -27.48‰ (against NBS-18, NBS-19 and NBS-22) and +1.5‰ (calibrated against IAEA N-1 and N-2) for C and N respectively.

The precision (1 σ) of the collagen results, based on the reproducibility of the gelatin standard measured six times within each run, was 0.09‰ for the first C run, 0.17‰ for

the second C run and 0.14‰ for both N runs. The precision of the plant bulk samples, based on the reproducibility of BROC-2 measured six times within each run, was 0.02 for was the C run and 0.09 for the N. Only those collagen samples that had a C:N ratio within the accepted range of 2.9-3.6 were included in the analysis (DeNiro 1985). All collagen samples were measured in triplicate except for sample KFO57, where enough material was available for only one measurement.

4.5. Charring correction

Experimental studies (Fraser et al. 2013a) have shown that charring increases plant $\delta^{15}N$ values by 1‰ (the species that were studied include *Triticum aestivum*, *Triticum monococcum*, *Tricium dicoccum*, *Triticum spelta*, *Hordeum vulage var. distichum and nudum*, *Lens culinaris*, *Pisum sativum*, *Vicia faba*). Even though Kanstrup et al. (2012) did not find any such charring offsets, their samples were only exposed to the experimental temperatures for 2 hours, which is too short to replicate the type of charring that causes the "optimal" morphological preservation like the one observed in the Kouphovouno samples (cf Charles et al. in prep). Following the findings of Fraser et al. (2013a), the plant $\delta^{15}N$ measurements obtained in this study were corrected for the charring effect by subtracting 1‰ from all normalized measurements.

4.6. ZooMS

Sheep and goat samples were distinguished on the basis of ZooMS (Zooarchaeology Mass Spectrometry). This method works on the basis of identifying species-specific markers in the amino acid sequences of collagen. The characteristic single peptide collagen markers have previously been identified for sheep and goat in a set of modern and archaeological specimen (Buckley et al. 2009, 2010). The same collagen that was

used to measure the stable isotopes in the present study was sequenced for ZooMS and all samples ran in triplicate. 62 out of 63 spots gave successful spectra.

5. Results and Discussion

5.1. Assessing the reliability of the crop isotope measurements

There are no set rules for accepting or rejecting plant isotope measurements based on their C:N ratios, like there are with collagen. In order to assess the reliability of the crop isotope measurements obtained in this study, we compared their C:N ratios to values of experimentally charred cereals and pulses (using data from Fraser et al. 2013a; %C values come from Supplementary Table 1, %N values come from Supplementary Table 2; see our Supplementary Table 2 for calculations of C:N ranges). The C:N ratios of the Kouphovouno cereals lie between 13.6 and 40.3; those of modern experimentally charred pulses are between 17.9 and 33.4. The C:N ratios of the Kouphovouno pulses range between 6.7 and 13.4; those of modern experimentally charred pulses are between s.8 and 13.1. Statistically, there are no significant differences between the two groups of values (two-tailed equal variance student's t-test between KFO cereals and modern charred pulses, p = 0.68; two-tailed equal variance student's t-test between KFO and %N measurements of the Kouphovouno samples show the same trends as the experimentally charred material:

1) pulses have higher %N than cereals, 2) cereals and pulses have indistinguishable %C values and 3) there is some species clustering in %N among the cereals (see Fig. 3). It is on the basis of the similarity of the C:N ratios and the trends apparent in the %C and %N values that we consider the isotope measurements reliable.

Insert Supplementary Table 2 here.

5.2. Crop δ^{15} N and soil growing conditions

Fig. 4 and Table 1 show that there is no overlap between the $\delta^{15}N$ values of the freethreshing wheat and hulled barley from Kouphovouno (mean $\delta^{15}N$ of free-threshing wheat (n=13) = 5.8 ± 0.7‰; mean $\delta^{15}N$ of hulled barley (n=7) = 2.7 ± 1.2‰). The only possible explanation for such a striking difference is that the crops were cultivated in different soils with distinct soil N properties. We will first consider the environmental and then the anthropogenic reasons for explaining the differences between the soils in the barley and the wheat fields.

None of the environmental factors discussed earlier are likely to have affected the fields around Kouphovouno:

- geophysical and hydrological reconstruction of the environment around Kouphovouno (Fouache et al. 2007) indicates that the soils in the nearby floodplains and alluvial fans were well drained and so it is unlikely that they were affected by soil salinity or denitrification
- the site is not located on the coast, so nitrates could not have been introduced to the land through sea-spray
- 3) an ecosystemic aridity effect would have affected the entire region and thus could not have elevated the δ^{15} N values of one field and not the other; furthermore, the measured Δ^{13} C values indicate that both cereals had enough water available to them (discussed below in section 5.4), and so it is unlikely that they suffered from different degrees of water stress

Out of the handful of anthropogenic factors that can cause ¹⁵N soil enrichment mentioned earlier, only one of them is likely to have affected the cultivated soils around

Kouphovouno. Burning of fossil fuels is not an issue for the Neolithic period and clearcutting of forests only produces a short-term enrichment. The most likely explanation is that the ¹⁵N enrichment in free-threshing wheat fields was caused by the application of farmyard manure, a phenomenon that has been found to raise the δ^{15} N of cultivated crops in several different experimental farm settings by up to 10‰ (Bogaard et al. 2007; Fraser et al. 2011; Kanstrup et al. 2011, 2013). The reason for the enrichment is that as animal dung releases gaseous ammonia through volatilization, the lighter ¹⁴N is preferentially lost to the atmosphere, leaving behind the heavier ¹⁵N, which is eventually taken up by the plants in the form of nitrates.

The application of kitchen waste material on cultivated fields can be considered as an extension of manuring, as decomposing material also provides ¹⁵N enrichment to the soil (Bogaard 2012). This practice can be detected with the presence of pottery sherds in areas of possible cultivated fields. Results of the land survey at Kouphovouno revealed a presence of Neolithic pottery on the top of the mound, and in smaller quantities on the slopes and the land adjacent to the tell (see Fig.4 in Cavanagh et al. 2004:83). Some of these sherds may have originated from kitchen waste. This raises the possibility that kitchen waste may have provided some opportunity for soil enrichment in the land proximal to the occupation areas; although the effect would not be as high as that provided by manure (cf Bogaard 2012).

Based on their experimental findings, Fraser et al. (2011) define three isotope ranges which represent three different levels of manure treatment:

- 1) long term high-level of manuring (values of 6‰ or above)
- 2) long term cultivation without manuring (values below 2.5‰)

 medium level of manuring resulting from either long-term cultivation with low manuring, residual effects after a period of intensive manuring or early years of a new cultivation regime (values between 2.5 and 6‰)

The manuring bands established experimentally may be used as a proxy for reconstructing farming practices in the Neolithic, but it is important to note that these bands can only be used for comparative purposes and not for assigning definite labels to any given measured values, as the absolute ratios vary in different climatic zones (due to a host of factors such as altitude and mean annual rainfall; van Klinken et al. 1994). In the absence of measurements of wild crop species, interpretations of the domestic crops measured in this study are confined to *inter-species differences* and can inform us about the differences in cultivation regimes rather than absolute environmental conditions.

Both cereal crops measured in this study fall into the medium manuring category, but the varying degree of ¹⁵N enrichment suggests that free-threshing wheat was manured more intensively than barley. This may reflect the farmers' awareness that barley can grow in more marginal conditions (Ceccarelli 1994; Ceccarelli et al. 2007; Guo et al. 2009), which may have been the basis for assigning differential value to the crops.

The experimentally manured crops used to establish the manuring bands are based on treatments of 20 – 35 tons of manure per hectare. Rowley-Conwy (1981) estimates that a pair of oxen can produce about 12 tons of manure per year. Combined with manure produced by other domestic animals kept on an intensive scale – goats, sheep, and pigs – it is conceivable that enough manure was available during the Neolithic to achieve similar levels of soil enrichment. Most of the manure would have needed to be applied manually on the fields, as letting the animals graze on the fields during an off-season

wouldn't provide the degree of enrichment implied by the isotope data. This suggests that the manuring strategy involved a very conscious and planned manipulation of the soil and was not simply a by-product of herding animals in the arable landscape. The intensive soil treatment attests to the long-term use of the fields, as it takes a number of years for the enriched N from manure to become available for uptake by crops. For this reason, it is unlikely that the crops were grown in a regime of shifting cultivation; rather, the same fields were used over a time-span of several generations on a schedule of rotation with pulses (to allow the soil to regenerate). As manuring leaves a long-term residual effect on the soil, these pulses would also be affected by the intensive soil treatment and evidence of this would provide more support for this hypothesis (see Section 5.3).

It is also possible that draught animals were used for spreading the manure on the fields and evidence of pathologies indicating the use of cattle for traction in the Middle Neolithic at Kouphovouno may support this view (Cantuel 2010, unpublished).

Two of the three Late Neolithic barley values are notably lower in δ^{15} N than the rest of the barley values (see Fig. 5). Even though the sample size is extremely limited, this may suggest that there was a shift towards reduced manuring of barley in the Late Neolithic.

Another way of inferring ¹⁵N enrichment in cultivated crops is by comparing the measured crop values to a local 'unmanured baseline' – a projection of the value of the forage consumed by local herbivores (cf Bogaard et al. 2013). The advantage of estimating this baseline is that it represents a diet composed of a mixture of wild plants, rather than individual measurements of possible wild plants, which would be the case if

wild plants were measured. In Kouphovouno, the mean domestic herbivore value (an average of the sheep, goats, cattle, and wild goat $\delta^{15}N$) is 4.9‰. Subtracting the approximate value of trophic enrichment of 5 ± 1‰ (see above) from the value of the local domestic herbivores gives a value of local forage of -0.1 ± 1‰. This figure is lower than the value of most of the measured crops, so this line of evidence corroborates the argument that both cereals grew in soils more enriched in ¹⁵N than the land on which the animals browsed/grazed. An exception are two barley samples from the Late Neolithic, which indicate reduced manuring of this crop in the later phase (see above).

5.3. Pulse δ^{15} N and soil growing conditions

Pulses are N₂-fixers that obtain most of their nitrogen from the atmosphere, and for this reason, they are less affected by soil ¹⁵N enrichment factors than crops such as cereals. The peas cultivated in Neolithic Kouphovouno (mean $\delta^{15}N = 1.3 \pm 0.3\%$) exhibit noticeable enrichment over their primary source of nitrogen: AIR, which is 0‰ (see Fig. 4). Low-intensity manuring of pulses is almost invisible in the isotope record as it causes increases in $\delta^{15}N$ which are almost indistinguishable from measurement error (Fraser et al. 2011). As a result, when there is a noticeable enrichment (an example is intensively manured farm-grown pulses in the island of Evvia (Fraser et al. 2011:2800, Fig.5), we are led to argue that the crops were grown under a high-intensity manuring regime.

5.4. Cereal Δ^{13} C and water status

Wheat and barley Δ^{13} C values (16.5 ± 0.5‰ and 18.4 ± 0.4‰ respectively) indicate that the water status of these two crops did not differ to a large extent. Based on controlled experiments on the effects of different watering regimes on the Δ^{13} C of modern cereals and pulses, Wallace et al. (2013) define three 'watering bands' that indicate the *broad approximations* of the water status of 'poorly watered', 'moderately watered' and 'well

watered' crops. Wheat grain in the 'moderately watered band' exhibits values between 16 and 17‰; those grains that have values higher than 17‰ fall into the 'well-watered band' and those that are lower than 16‰ are grouped into the 'poorly watered band'. The bands for barley have been defined by adding a mean offset of 1.5‰ to the wheat bands, owing to a known physiological offset between the two crops (Araus et al. 1997, 1999; Jiang et al. 2006; Wallace et al. 2013).

Both cereal crops measured in this study fall into the 'moderately watered band' (see Fig. 6) and prompt us to suggest that both crops had sufficient amounts of water available to them. The fact that barley is situated slightly higher within its watering band than wheat should not be considered significant, because the boundaries of the barley band have uncertainty attached to them, owing to the fact that the physiological offset between wheat and barley (used to define the barley bands) has been variably reported as lying between 1 and 2‰ (Araus et al. 1997, 1999; Jiang et al. 2006). In addition, Wallace et al. (2013) argue that crops grown under the same regime may have Δ^{13} C variable by ± 0.5-1‰. What is clear from the present data is that both cereals received sufficient amounts of water during their growth.

5.5. Pulse Δ^{13} C and water status

The pulses, represented by peas and a single sample of lentil, exhibit a clearly distinct water status compared to the cereals. The watering bands for pulses defined through Wallace et al.'s (2013) controlled experiments have similar values to those of wheat grain: 'moderately watered pulses' lie between 16 and 17‰, 'highly watered pulses' are situated above 17‰ and 'poorly watered pulses' fall below 16.

The mean Δ^{13} C of the peas (18.9 ± 0.9‰) and the value of the lentil (19.1‰) (Fig. 6) lie c. 2‰ above the well-watered pulse band. Experimental studies have shown that there are no obvious trends with seasonality and Δ^{13} C, only that pulses seem to be more sensitive to water input than cereals (pulses grown in dry soils look 'very dry' and pulses grown in wet soils look 'very wet') (Wallace et al. 2013). The effect this sensitivity would have on the pulse Δ^{13} C, however, would not be as great as to account for the high Δ^{13} C values measured in this study. For this reason, we argue that the pulses were watered artificially.

The higher standard deviation of the peas (0.9‰ compared 0.5‰ and 0.4‰ of the cereals) may indicate that the pulses were being watered by hand, a practice that has been suggested as a possible cause of high Δ^{13} C variability in archaeobotanical remains from Assiros Toumba (Wallace 2011, unpublished). There is no archaeological evidence for the use of irrigation at Kouphovouno. In modern collections, such higher Δ^{13} C variability was observed at a small-scale 'traditional farm' situation on the Greek island of Evvia, where the application of water was not standardized among different farmers (Wallace et al. 2013).

Thus, both the carbon and nitrogen lines of evidence suggest that pulse crops were being cultivated on an intensive, likely 'garden type' scale. Note that even though there is a slight suggestion that in the Late Neolithic, the δ^{13} C of peas increased (see Fig. 5) (and therefore Δ^{13} C decreased), all the peas still fall well above the moderately watered band.

5.6. Animal $\delta^{13}C \& \delta^{15}N$ and diets

The δ^{13} C and δ^{15} N values of the animal bone collagen reflect patterns that conform to general expectations, but also enable us to look at more subtle differences in the diets of the Neolithic livestock (see Fig. 4 and Table 2). Sheep, goats and cattle all cluster in the same region, which is a result of their herbivorous nature. More subtle differences in the feeding strategies of the sheep and goat in the Middle and Late Neolithic periods are discussed below in section 5.8. The values of the pigs and the dogs are more elevated on the δ^{15} N scale, which is in line with their more omnivorous dietary habits. The δ^{13} C of the dogs is less negative than that of the pigs, which may be the result of higher consumption of meat products (different authors report offsets of +1-2.6‰ for the 'carnivore effect': Bocherens et al. 1995; Lee-Thorp et al. 1989; Sillen et al. 1989). The same is the case for the one value of bear. Part of the reason for the high δ^{15} N of the pigs may be that they consumed kitchen waste, which may have contained the grains or by-products of manured crops (discussed below in section 5.7). The one value of cattle with extremely elevated δ^{15} N is considered to be an outlier.

5.7. Projected chaff $\delta^{13}C$ & $\delta^{15}N$ and pig foddering

Measurement of plant isotope values enables us to not only infer ancient crop growing conditions, but also to evaluate their potential contributions to the human and animal diets. Having measured three different crop types cultivated at Kouphovouno, we are now better equipped to try to interpret which of the crops *may* have been consumed by the domestic animals.

Experimental studies have shown that there is a c. -2.4‰ offset in δ^{15} N and -1.9‰ in wheat δ^{13} C and -1.7% in barley δ^{13} C between cereal grain and rachis (the stem within the cereal ear) (Fraser et al. 2011; Wallace et al. 2013). Fig. 7 shows the projected values of the free-threshing wheat and barley chaff for the Kouphovouno crops (wheat

chaff: δ^{15} N $\approx 3.5\%$, δ^{13} C $\approx -23.8\%$; barley chaff: δ^{15} N $\approx 0.3\%$, δ^{13} C $\approx -25.6\%$). Among all the animals studied, pigs are the ones that would have obtained most of their food from in or around the village (Rivals et al. 2011). Subtracting the diet-tissue spacing value of 5 $\pm 1\%$ (see above) from the measured δ^{15} N of the pigs (mean $5.9 \pm 0.9\%$), we get a value of c. $0.9 \pm 1\%$ for the composition of the pig diets. This is consistent with a diet made up of a mixture of barley grain and/or by-product, a small contribution from the byproduct of wheat, any component of the pulses, or other vegetation with comparable isotopic composition. What the pigs could not have been consuming to a significant extent is the free-threshing wheat grain. This result suggests that wheat was grown exclusively for human consumption and thus had a different cultural value to barley in this Neolithic context.

5.8. Sheep & goat diets and diachronic change

Sheep and goats are often grouped into one domestic animal category due to the morphological similarity of their skeletons, but the two species exhibit distinct grazing/browsing adaptations and are thus expected to have different diets. In this study, the differentiation of sheep and goat bones using ZooMS analysis allowed us to investigate the very nature of those differences.

The two major factors to consider while differentiating between sheep and goat diets on an isotopic level are: 1) the type of habitat in which the animals graze/browse, 2) the choice of plants or plant parts that they consume; and these two factors may be influenced by the sizes of the animal herds. Sheep prefer to graze on grassy vegetation in open habitats while goats favor more leafy and woody vegetation in closed environments. If kept in large numbers, the animals are likely herded separately and taken to their optimal habitats (Halstead pers.comm.; Shipley 1999). Their diets thus

reflect both habitat and plant choices. If kept in smaller numbers, the sheep and goat are likely herded together in the same pasture areas or on parts of the arable landscape (Halstead 2006; Halstead pers. comm.). Differences in their isotope values are thus mostly influenced by the plant part choices made by each species.

Fig. 8a shows that sheep and goat at Neolithic Kouphovouno exhibit isotopically diverging diets, and further that these differences changed between the Middle and the Late Neolithic. In the Middle Neolithic, the diets of these animals differed on the δ^{13} C scale (this difference is statistically significant at the 99% confidence level; two-tailed equal variance student's t-test, p = 0.0041), while in the Late Neolithic, they differed on the δ^{15} N scale (the differences are statistically significant at 95% confidence; two-tailed equal variance student's t-test, p = 0.043). Mean δ^{13} C of MN sheep (n=5) = -20.8 ± 0.2‰; mean δ^{13} C of MN goats (n=3) = -20.1 ± 0.1‰. Mean δ^{13} C of LN sheep (n=7) = -20.3 ± 0.4‰; mean δ^{13} C of LN goats (n=4) = -20.1 ± 0.3‰). Mean δ^{15} N of LN sheep = 5.3 ± 0.7‰; mean δ^{15} N of LN goats = 4.2 ± 0.9‰; mean δ^{15} N of MN goats = 5.0 ± 1.0‰.

Middle Neolithic sheep and goats at Kouphovouno have similar δ^{15} N, but the sheep have lower δ^{13} C than the goats. Woody vegetation tends to have lower δ^{13} C values due to its higher content of lignin (see Svendsen (2001) on lignin composition of browse and Benner et al. (1987) on the carbon isotopic composition of lignin), although several factors are likely to determine the differences between grass and leaf δ^{13} C in the same environment (see Escudero et al. (2008) for a study on how leaf longevity is related to water availability, photosynthetic rates and δ^{13} C in woody plants). If the assumption that δ^{13} C of woody vegetation in the Sparta basin was lower than that of grass is correct and the difference in δ^{13} C of the sheep and goats was due to the choice of plants, goats

would be expected to have lower δ^{13} C values. As this is not the case, the isotopic distinctions are likely a result of feeding in different habitats.

There are two types of habitats which could produce more ¹³C depleted grasses for the sheep to consume. The first are forested environments affected by the canopy effect. This phenomenon has been argued to cause depletion in ¹³C in the understories of forest canopies (as opposed to leaves/parts of trees situated higher up or vegetation growing in more open environments) due to a combination of two factors: i) the reassimilation of recycled and ¹³C-depleted CO₂ from respiration and/or decomposition of forest litter, and ii) fractionation caused by reduced light intensity in the lower parts of forests during photosynthesis (Bonafini et al. 2013; Drucker et al. 2008; Heaton 1999; van der Merwe and Medina 1991).

The second type of habitat where the Middle Neolithic sheep could have obtained their ¹³C depleted grasses are areas with wetter soils (cf Farquhar et al. 1982, 1989; Wallace et al. 2013). As sheep prefer to subsist in more open environments rather than forested canopies, this latter explanation is more likely. The distinction in diets between the MN ovicaprids may be the result of large enough herd sizes, which justified the management of sheep and goat in different habitats. The one sample of goat measured, which comes from the Middle Neolithic, has a less negative δ^{13} C than all the MN sheep and goat (see Fig. 8a). This suggests that it was not exploiting either of the habitats that the sheep and goat were subsisting in. The limited sample sizes (3 MN goats, 4 LN goats, 5 MN sheep and 7 LN sheep) remind us that we must exercise caution with interpreting these results, but the discussion presented herein is only concerned with statistically significant differences. Still, the main aim of this part of the discussion is to demonstrate the

avenues of research that can be pursued with this type of stable isotope data, rather than to provide conclusive answers to the archaeological questions.

In the Late Neolithic, the isotopes of the sheep and goats are variable on the $\delta^{15}N$ scale, while the $\delta^{13}C$ are indistinguishable (but both $\delta^{13}C$ and $\delta^{15}N$ values are more variable on an intra-species level). In light of the feeding strategies discussed above, it is possible that in the later part of the Neolithic, the sizes of the sheep and goat herds decreased and both animals were now being managed together and the slight differences in their $\delta^{15}N$ differed were caused by the choice of plants and plant parts. The famers may have made greater use of the arable land for grazing during a fallow season, and this would be consistent with Halstead's (2000) model of intensive mixed farming, where small flocks are managed within the interstices of the cultivated land.

Beyond informing us about the possible distinctions in the feeding strategies of the Neolithic sheep and goat, the results presented herein indicate that there was a shift in the management strategy of the ovicaprids between the two Neolithic phases. This shift may have manifested itself with a decrease in the size of the sheep herds, leading to a reduced predominance of sheep in the livestock population. A similar shift has been observed more broadly by Halstead (2000) between the Early and the Late Greek Neolithic: in the Early Neolithic, faunal assemblages were dominated by sheep, while Late Neolithic assemblages show a more balanced representation of all the domestic animals.

The changes in animal management at Kouphovouno happened in the context of shifts in ceramic styles and expression of socio-cultural identity. In the Middle Neolithic, the pottery style was more uniform and greater emphasis was placed on expression of

group identity, while in the Late Neolithic, a more diversified pottery style signaled a movement towards greater individuality (Mee et al. in prep.). At the same time, different livestock management strategies may have been fueled by a changed attitude towards land ownership.

5.9. Cattle & dog diets and environmental change?

The results discussed so far indicate that there was a diachronic change in the feeding strategies of the sheep and goats. Conversely, Fig. 8b shows that the diets of pigs did not change through time (the differences are not statistically significant: two-tailed equal variance student's t-test for the δ^{13} C values, p = 0.11; two tailed equal variance student's t-test for the δ^{15} N values, p = 0.95). A closer look at the other major domestic animals, cattle and dogs, add to the story about change in animal management strategies in the Late Neolithic.

All the Late Neolithic dogs have more negative δ^{13} C values than all the Middle Neolithic dogs (δ^{13} C of MN dogs (n=3) = -18.6 ± 0.5‰; δ^{13} C of LN dogs (n=4) = -19.6 ± 0.1‰) (see Fig. 8c; two-tailed equal variance student's t-test shows significance at the 99% confidence level, p = 0.0088). Their δ^{15} N values are indistinguishable (two-tailed student's equal variance t-test, p = 0.58). As the bulk of dog diet is not composed of just grasses and leaves but of a more diverse range of food sources which have undergone further fractionation after being consumed by primary herbivores, their δ^{13} C do not reflect the water status of the areas from which their foods were derived. Thus, the lower δ^{13} C values cannot be a result of subsisting on food items grown in wetter soils (as is the case with Middle Neolithic sheep).

The diachronic difference in δ^{13} C could be the result of reduced consumption of meat products in the Late Neolithic in conjunction with an increased consumption of cultivated crops. Meat products have a positive effect on both δ^{13} C and δ^{15} N (for the carnivore effect in δ^{13} C see Bocherens et al. 1995; Lee-Thorp et al. 1989; Sillen et al. 1989; for trophic level enrichment in δ^{15} N see DeNiro and Epstein 1981; Hedges and Reynard 2007; Minagawa and Wada 1984). Consumption of less meat in the Late Neolithic would decrease the dogs' δ^{13} C and δ^{15} N values, but an increased consumption of manured crop products would compensate for the decrease in δ^{15} N so in the end, only the effect on carbon would be detectable. The crop products could have been obtained by scavenging on human food waste. Note that this suggestion does not exclude the possibility of meat consumption in the LN altogether. It is still likely that dogs consumed more meat than pigs due to their less negative δ^{13} C values, but what this discussion centers on (once again taking the limited sample size into account) is the likelihood that in the Late Neolithic, the dogs consumed it to a *smaller extent than in the Middle Neolithic*.

The browsing and grazing adaptations of herbivores exist on a continuum, in a way that the animals pick and chose the most nutritious food that is available, whether it be grass or browse (Lisa Shipley, pers.comm.). For this reason, there is a lot of overlap between the dietary choices of sheep, goat and cattle. There is a statistically significant difference at the 95% confidence between the δ^{15} N values of the Middle Neolithic cattle (n=4) and Late Neolithic cattle (n=11) (two-tailed equal variance student's t-test, p = 0.010). Despite the limited sample size, we will attempt to explain what such a difference may mean. As with the ovicaprids, the feeding strategy of the cattle changed in the Late Neolithic (see Fig. 8d). The Middle Neolithic cattle are situated in the same δ^{13} C region as the Middle Neolithic sheep (two-tailed equal variance student's t-test, p = 0.40) and

this may be because they shared the same wetter pasture grasses, perhaps along the banks of the Eurotas river. In the Late Neolithic, the cattle seems to divide into two clusters; one that overlaps with the Middle Neolithic sheep and one that does not (see Fig. 8d). This may mean that while the same pasture grasses were still consumed by cattle in the Late Neolithic, some cattle may have been kept in other, drier, areas as well. The cattle that may have grazed along the banks of the Eurotas also exhibit quite a wide range of δ^{15} N values, which includes the lowest δ^{15} N value of all the domestic animals at Kouphovouno. This may be related to consumption of a wide range of different grasses in this habitat.

Most of the domestic animals apart from pigs seem to have experienced a shift in feeding strategies between the Middle and the Late Neolithic. On the isotopic scale, these shifts did not move in the same direction. The dogs' δ^{13} C decreased, the sheep δ^{13} C increased, the goats' and cattle' δ^{15} N decreased while the sheep δ^{15} N increased. This suggests that the changes were not the result of some environmental change (one that would cause an increase in δ^{15} N in all Late Neolithic vegetation, for example), but that the changes were related to shifts in management of the farm animals.

6. Synthesis and conclusion

This study is one of the first to integrate plant and animal dietary isotopes (see also Fraser et al. (2013b) and Lightfoot and Stevens (2012)) and the first to combine this methodology with collagen sequencing of sheep and goats. The data provide support for the model of small-scale mixed agriculture and settlement organization put forth by previous archaeobotanical and archaeozoological investigations (Bogaard 2004a, 2004b, 2005; Bogaard and Isaakidou 2010; Halstead 1996, 2000, 2006; Kotsakis 1999).

Beyond providing support to this theory, however, the stable isotopic method offers new insight for understanding the integration of the crop cultivation and animal husbandry and how these regimes functioned within the given settlement organization over the long-term. Some of the interpretations in this study are limited by small sample sizes, and their primary value is in demonstrating what sorts of questions this methodology can address rather than for providing definite answers for the prehistoric farming techniques.

Even though the tell at Kouphovouno was not excavated in its entirety and we therefore do not know how dense the settlement was, the available archaeology indicates that the village was nucleated or organized into neighborhoods. Such settlement configuration would have provided opportunity for intensive agriculture either on the periphery of the settlement and/or in plots adjacent to the neighborhoods. The more proximal fields would have been more valuable as they provided the opportunity for high laborinvestment under a small-scale intensive treatment (cf Jones et al. 1999). This is where the crops grown for human consumption – free-threshing wheat and pulses – were likely grown in rotation with one another, receiving manure in the form of dung from the domestic animals.

In the Late Neolithic, the application of manure may have been less labor-intensive. The few LN barley samples indicates that this crop likely received less manure than it did during the Middle Neolithic and the wheat/pulse fields may have been manured directly by sheep and goat grazing on the arable plots. Furthermore in this Neolithic phase, the scale of sheep/goat management seems to have decreased, dogs fed on smaller amounts of meat products and larger amounts of cultivated crops and cattle were likely taken to two different areas for grazing. These diachronic shifts in crop and animal management highlight the fact that even though small-scale mixed farming embraced

the "Neolithic package" in a seemingly consistent fashion across the continent, it did allow for enough flexibility for changes in the scale of crop and animal husbandry and the amount of labor-investment.

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List of figure captions

Fig. 1 Geographical location of Kouphovouno in Laconia, Peloponnese (prepared by Jean Cantuel, from Rivals et al. 2011)

Fig. 2 Plan of the archaeological excavations at Kouphovouno undertaken between 2001 and 2007

Fig. 3 %C and %N values of experimentally charred and uncharred cereals and pulses compared to free-threshing wheat, hulled barley and peas from Kouphovouno (KFO). The experimental cereals (circled) include bread wheat, einkorn wheat, emmer wheat, hulled barley and naked barley. The experimental pulses (circled) include peas, lentils and broad beans (data comes from Fraser et al. 2013a)

Fig. 4 δ^{15} N and δ^{13} C of all bulk plant and animal collagen samples from Neolithic Kouphovouno

Fig. 5 δ^{15} N and δ^{13} C of bulk samples of Middle Neolithic (MN) and Late Neolithic (LN) crops from Kouphovouno

Fig. 6 Δ^{13} C of bulk plant values from Neolithic Kouphovouno (the values were converted from δ^{13} C following the principles outlined by Farquhar et al. 1989 and Ferrio et al. 2005, see text). Measured values are plotted with watering bands established by Wallace et al. (2013)

Fig. 7 Animal collagen and bulk plant $\delta^{15}N$ and $\delta^{13}C$ values with projected values of chaff for free-threshing wheat and hulled barley, assuming a $\delta^{15}N$ offset of -2.4‰ for of both cereals and $\delta^{13}C$ offset of -1.9‰ for wheat and -1.7‰ for barley; following Fraser et al. (2011) and Wallace et al. (2013)

Fig. 8 δ^{15} N and δ^{13} C of Middle Neolithic (MN) and Late Neolithic (LN) (a) sheep and goat, (b) pigs, (c) dogs and (d) cattle.

 Table 1
 Stable isotope results of Neolithic bulk crop samples from Kouphovouno

Table 2 Stable isotope results of Neolithic faunal samples from Kouphovouno

Supplementary Table 1 Details of the archaeological contexts from Kouphovouno sampled for stable isotope analysis

Supplementary Table 2 %C, %N and C:N values for Kouphovouno cereals and pulses and for experimentally charred modern cereals and pulses (the latter come from Fraser et al. 2013a). See Section 2 in Results and Discussion on how the C:N ranges were used to assess the reliability of the plant isotope measurements in this study.

Lab code	Area/ context	Phase (Middle Neolithic/Late Neolithic)	δ¹³C	δ ¹⁵ N ^a	%C	%N	C:N ^b	δ^{13} C of CO ₂ ^c	Δ ¹³ C ^d
Free-thres	hing wheat	(n=13)							
KFO3	C0266	MN	-22.4	4.9	46.9	2.8	19.6	-6.6	16.2
KFO5	C0295	MN	-22.3	7.1	48.1	3.2	17.5	-6.6	16.1
KFO9	C0800	MN	-23.4	5.4	49.0	3.1	18.3	-6.6	17.2
KFO10	C0804	MN	-22.5	5.8	51.3	2.9	20.6	-6.6	16.2
KFO11	C0806	MN	-22.2	5.6	40.1	3.4	13.6	-6.6	16.0
KFO12	C0812	MN	-23.3	6.6	36.0	2.2	19.5	-6.6	17.0
KFO13	C0825	MN	-23.0	7.0	51.5	2.9	20.4	-6.6	16.8
KFO16	C0905	MN	-22.3	5.2	40.1	2.9	16.2	-6.6	16.1
KFO18	C0911	MN	-22.3	5.5	46.2	2.8	19.2	-6.6	16.1
KFO19	C0918	MN	-22.5	5.9	50.8	3.1	19.0	-6.6	16.2
KFO2	B0185	LN	-23.4	5.9	48.6	3.1	18.3	-6.4	17.4
KFO6	G1-1624	LN	-22.4	5.4	56.1	3.1	21.0	-6.4	16.4
KFO7	G1-1627	LN	-23.0	5.5	50.6	2.8	21.3	-6.4	17.0
	i	average all wheat	-22.7	5.8					16.5
	stanc	lard devision (1 σ)	0.4	0.7					0.5
	a	verage MN wheat	-22.6	5.9					16.4
	stanc	lard devision (1σ)	0.4	0.8					0.4
	a	verage LN wheat	-22.9	5.6					16.9
	stanc	lard devision (1σ)	0.5	0.3					0.5
Hulled bar	'ley (n=/)								
KEOA	00066	MANI	24 E	2.4	40.0	<u>.</u>	25.2	6.6	10.0
	C0266		-24.5	3.1	49.0 54.2	2.3 2.2	20.0	-0.0	10.3
KFO14	C0005		-24.2	4.2	54.5	2.3	27.4	-0.0	10.0
KFO15	C0905		-24.5	2.7	50.Z	2.0	29.1	-0.0	10.3
KEO1	B0134		-24.4	0.7	44.1	2.2	25.1	-0.0	10.2
KEO20	G1_2003		-24.0	27	45.4	2.0	25.7	-6.4	10.4
KFO20	G1-2003		-24.4 25.1	2.7	40.1 56.0	2.0	20.1 40.2	-0.4	10.4
Ri Oo	110703	LIN	-23.1	1.0	50.0	1.0	40.5	-0.4	19.2
	;	average all barley	-24 5	27					18.4
	stanc	lard devision (1σ)	0.3	12					0.4
	a	verage MN barley	-24.4	3.4					18.2
	stand	lard devision (1σ)	0.1	0.6					0.1
	a	verage LN barley	-24.7	1.7					18.7
	stanc	lard devision (1σ)	0.4	1.0					0.5
Pea (n=7)			-	-					
KFO24	C0844	MN	-26.1	0.9	48.2	6.0	9.3	-6.6	20.1
KFO27	C0905	MN	-25.9	1.6	50.4	4.4	13.4	-6.6	19.8
KFO28	C0918	MN	-25.1	1.3	28.1	3.6	9.1	-6.6	19.0
KFO29	C1024	MN	-24.4	1.1	45.3	4.5	11.8	-6.6	18.2
KFO21	B0134	LN	-24.8	1.4	46.6	4.3	12.6	-6.6	18.7
KFO22	G1-1666	LN	-23.4	1.2	54.1	5.3	11.9	-6.4	17.4
KFO23	H0709	LN	-24.8	1.7	39.4	6.8	6.7	-6.4	18.8
1									

	i	average all peas	-24.9	1.3				18.9
	standa	ard devision (1 σ)	0.9	0.3				0.9
	a	verage MN peas	-25.4	1.2				19.3
	standa	ard devision (1 σ)	0.8	0.3				0.9
	a	verage LN peas	-24.3	1.4				18.3
	standa	ard devision (1 σ)	0.8	0.3				0.8
Lentil (n=1)								
KFO25	C0850	MN	-25.2	-	33.2	-	-6.6	19.1

^a All reported plant δ^{15} N values have been corrected for the charring effect by subtracting 1‰ (cf Fraser

et al. 2013) ^b calculated using %C from the δ^{13} C measurement and %N from the δ^{15} N measurement; using equation: (%C/%N)*(14/12)

^c average value for the chronological time period

^d calculated using AIRCO2_LOESS data calibrator (Ferrio et al. 2005)

		Phase (Middle					
Lah no	Area/	Neolithic/Late	δ ¹³ C	δ ¹⁵ N	%	%N	C·N
Bos taurus (n = 1	5)	(Contril)	00	0 11	700	7014	0.11
KOUP-26	C1705	MN	-21.0	5.4	22.1	7.3	3.5
KOUP-27	C1705	MN	-17.7	8.4	33.7	11.7	3.4
KOUP-30	C1705	MN	-20.7	5.1	26.0	8.7	3.5
KOUP-52	C1713	MN	-20.6	5.6	25.7	9.1	3.3
KOUP-57	G1-2000	LN	-19.8	4.8	35.7	12.7	3.3
KOUP-58	G1-2000	LN	-21.0	3.8	32.4	11.9	3.2
KOUP-68	G1-2001	LN	-20.9	4.3	37.1	13.0	3.3
KOUP-69	G1-2001	LN	-20.9	4.9	32.7	11.5	3.3
KOUP-70	G1-2001	LN	-20.3	4.8	36.1	12.7	3.3
KOUP-71	G1-2001	LN	-20.9	5.2	38.7	13.8	3.3
KOUP-75	G2-1102	LN	-20.1	4.8	42.0	14.1	3.5
KOUP-84	G2-1115	LN	-19.7	5.2	38.2	13.4	3.3
KOUP-93	G2-1136	LN	-20.8	3.9	24.6	8.0	3.6
KOUP-96	G2-1139	LN	-21.0	2.7	40.2	13.5	3.5
KOUP-97	G2-1139	LN	-21.1	3.9	39.7	13.7	3.4
		average all cattle	-20.4	4.9			
	s	standard devision (1 σ)	0.9	1.2			
		average MN cattle	-20.0	6.1			
	S	standard devision (1σ)	1.5	1.5			
		average LN cattle	-20.6	4.4			
	S	standard devision (1σ)	0.5	0.8			
Canis (n = 7)							
KFO51	C1705	MN	-19.1	7.3	38.9	13.8	3.3
KFO52	C1705	MN	-18.3	7.8	40.6	14.3	3.3
KFO53	C1705	MN	-18.3	7.6	39.7	14.2	3.3
KFO65	G1-2000	LN	-19.6	7.8	41.1	14.8	3.3
KFO68	G1-2001	LN	-19.6	6.8	44.3	15.4	3.4
KFO74	G2-1132	LN	-19.4	8.1	38.9	14.0	3.2
KFO76	G2-1136	LN	-19.9	8.7	34.4	12.7	3.2
		average all dog	-19.2	7.7			
	S	tandard devision (1σ)	0.6	0.6			
		average MN dog	-18.6	7.6			
	S	tandard devision (1σ)	0.5	0.3			
		average LN dog	-19.6	7.9			
	S	standard devision (1σ)	0.2	0.8			
Ovis aries (n = 12))						. .
KOUP-13	C1705	MN	-20.8	5.2	35.9	12.4	3.4

KOUP-16	C1705	MN	-20.6	4.2	34.1	11.7	3.4
KOUP-39	C1713	MN	-20.5	4.8	24.7	8.9	3.2
KOUP-41	C1713	MN	-21.1	5.3	32.0	11.5	3.2
KOUP-42	C1713	MN	-20.8	4.6	40.0	14.2	3.3
KOUP-65	KOUP-65 G1-2001 LN		-20.6	6.1	32.0	11.7	3.2
KOUP-66	G1-2001	LN	-20.6	4.7	40.9	14.1	3.4
KOUP-67	G1-2001	LN	-19.6	5.1	38.4	13.4	3.4
KOUP-79	G2-1103	LN	-20.0	6.3	40.8	14.3	3.3
KOUP-81	G2-1104	LN	-20.9	5.4	39.0	13.2	3.5
KOUP-83	G2-1115	LN	-20.2	4.4	16.6	5.8	3.3
KOUP-92	G2-1136	LN	-20.2	5.1	40.3	13.2	3.6
		average all sheep	-20.5	5.1			
	st	tandard devision (1 σ)	0.4	0.6			
		average MN sheep	-20.8	4.8			
	st	tandard devision (1 σ)	0.2	0.4			
		average LN sheep	-20.3	5.3			
	St	tandard devision (1 σ)	0.4	0.7			
Capra (n = 7)							
KOUP-12	C1705	MN	-20.2	4.9	31.2	10.6	3.5
KOUP-14	C1705	MN	-20.1	6.0	38.2	13.2	3.4
KOUP-37	C1713	MN	-20.1	4.0	35.1	12.4	3.3
KOUP-59	G1-2000	LN	-20.1	5.4	33.8	11.9	3.3
KOUP-60	G1-2000	LN	-19.7	4.1	35.7	12.6	3.3
KOUP-64	G1-2001	LN	-20.1	3.3	35.1	12.9	3.2
KOUP-88	G2-1132	LN	-20.5	3.9	37.6	13.3	3.3
		average all goat	-20.1	4.5			
	st	tandard devision (1 σ)	0.2	1.0			
		average MN goat	-20.1	5.0			
	st	tandard devision (1 σ)	0.1	1.0			
		average LN goat	-20.1	4.2			
	st	tandard devision (1 σ)	0.3	0.9			
Sus domesticus	(n = 23)						
KFO44	C1705	MN	-20.7	5.0	45.5	15.6	3.4
KFO45	C1705	MN	-20.5	5.3	39.9	14.3	3.3
KFO46	C1705	MN	-20.0	6.6	38.1	13.0	3.5
KFO48	C1705	MN	-19.8	7.4	40.4	14.1	3.4
KFO49	C1705	MN	-20.0	6.3	41.1	14.5	3.3
KFO50	C1705	MN	-20.2	6.9	39.5	13.9	3.3
KFO54	C1710	MN	-20.5	7.0	39.9	14.3	3.3
KF055 C1713 MN		-20.3	4.9	37.8	13.9	3.2	

KFO56	C1713	MN	-20.2	5.0	43.1	15.0	3.4
KFO57	C1713	MN	-20.3	5.2	38.3	13.3	3.4
KFO58	C1713	MN	-20.1	5.6	42.8	15.2	3.3
KFO59	C1713	MN	-20.0	5.0	38.6	14.0	3.2
KFO60	C1713	MN	-19.1	6.3	40.3	14.6	3.2
KFO61	G1-2000	LN	-20.1	6.0	40.3	14.4	3.3
KFO62	G1-2000	LN	-20.0	6.2	41.5	14.9	3.3
KFO63	G1-2000	LN	-20.6	5.8	42.7	15.3	3.3
KFO66	G1-2001	LN	-21.1	4.4	39.9	14.3	3.3
KFO67	G1-2001	LN	-19.6	6.4	42.1	14.9	3.3
KFO69	G2-1103	LN	-21.0	7.5	36.5	12.7	3.4
KFO71	G2-1104	LN	-20.7	4.6	38.1	13.4	3.3
KFO72	G2-1132	LN	-20.5	6.4	45.2	14.9	3.5
KFO73	G2-1132	LN	-20.3	6.7	38.9	14.2	3.2
KFO75	G2-1136	LN	-20.4	5.1	38.1	14.1	3.2
		average all pig	-20.3	5.9			
	S	tandard devision (1σ)	0.4	0.9			
		average MN pig	-20.1	5.9			
	S	tandard devision (1σ)	0.4	0.9			
		average LN pig	-20.4	5.9			
	S	tandard devision (1σ)	0.5	1.0			
Sus scrofa (n=1)							
KFO47	C1705	MN	-21.4	3.9	41.0	14.3	3.4
Lepus (n=1)							
KOUP-53	C1713	MN	-21.8	2.4	35.4	13.4	3.1
Ursus (n=1)							
KFO64	G1-2000	LN	-19.7	8.3	37.2	13.8	3.2
Capra sp. (n=1)							
KOUP-38	C1713	MN	-19.7	4.2	17.4	6.4	3.2
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Highlights

- integrated stable isotope analysis of crops and animals reveals nuanced husbandry practices
- Middle and Late Neolithic crops were manured and watered to varying degrees
- herding practices varied among species
- > diachronic changes in faunal isotopes reflect management shifts
- > this study reveals the sophisticated character of early mixed farming

Context number	Context type	Date	Relation to other contexts	Number of stable isotope samples	Samples deriving from this context
AREA C					
C1024	destruction layer consisting of stones, clay and fragments of burnt building clay lying on top of a burnt floor	Middle Neolithic	immediately below plough soil C1001/C1704	1 plant	KFO29
C1705	upper part of a probable dump rich in pottery and animal bone	Middle Neolithic	lay immediately north of the north wall of Structure C1; lay below plough soil C1704	17 faunal	KOUP-12, KOUP-13, KOUP-14, KOUP-16, KOUP-26, KOUP-27, KOUP-30, KFO44, KFO45, KFO46, KFO47, KFO48, KFO49, KFO50, KFO51, KFO52, KFO53
C1713	lower part of a probable dump with patches of clay with charcoal inclusions	Middle Neolithic	pre-dates C1705	13 faunal	KOUP-37 KOUP-38, KOUP-39, KOUP-41, KOUP-42, KOUP-52, KOUP-53, KFO55, KFO56, KFO57, KFO58, KFO59, KFO60
C0295	top layer of a later Middle Helladic grave fill, the fill consisted solely of MN pottery	Middle Neolithic	the grave also cut through contexts C0905, C0911 and C0918	1 plant	KFO5
C0266	floor of Structure C4, most likely an outside working area with a saddle quern and a cache of charred seeds	Middle Neolithic	same context as C0905 excavated in a different year	2 plant	KFO3, KFO4
C0905	floor of Structure C4, most likely an outside working area with a saddle quern and a cache of charred seeds	Middle Neolithic; AMS date of a free- threshing wheat grain from this context: 5613-5478 cal BC	same context as C0266 excavated in a different year; set above C0911	3 plant	KFO15, KFO16, KFO27

C0911	destruction layer	Middle Neolithic; AMS	pre-dates C0905 and post-	2 plant	KFO17, KFO18
		date of a free-	dates C0918		
		from this context.	C		
		5664-5546 cal BC		Y	
C0918	floor of structure C4 containing	Middle Neolithic; AMS	pre-dates C0911	2 plant	KFO19, KFO28
	chipped stone and a large	date of a free-			
	quern (still in use with floor	threshing wheat grain			
	C0905)	from this context: 5615-5483 cal BC			
C1710	stone base of the south wall of	Middle Neolithic	pre-dates construction of	1 faunal	KFO54
	Structure C II		floors of the structure		
			which are likely		
			and C0918		
C0800	fill	Middle Neolithic	pre-dates C0806 and	1 plant	KFO9
C0806	liping of pit C0804	Middle Neolithic	Liping of pit C0804	1 plant	KEO11
C0804	fill of amoll pit containing	Middle Neolithia	nning of pit C0804	1 plant	KFO10
0004	chipped stone, animal bones		consists of material lining	i piant	KFU10
	and stones		the pit		
C0812	levelled surface consisting of	Middle Neolithic	pre-dates C0918	1 plant	KFO12
	occupation debris used to				
	make a floor foundation				
C0825	foundation packing for the floor	Middle Neolithic	pre-dates C0812	1 plant	KFO13
	of a surface rich in charcoal				
C0844	building debris forming a	Middle Neolithic; AMS	pre-dates C0825	1 plant	KFO24
	relatively flat foundation on	date of an			
	which C0825 was placed	indeterminate wheat			
		F716 F574 col PC			
C0850	dumped material with frequent	Middle Neolithie	pro datas C0844	1 plant	KEO25
00000	inclusions of charcoal and		pie-uales Cuo44		NFU20
	oxidised clay				

C0854	the bottom layer of sounding C containing frequent charcoal inclusions, situated above natural sediment	Middle Neolithic; AMS date of hulled barley grains from this context: 5843-5673 cal BC	pre-dates C0850	1 plant	KFO14
Area G1		·		/	÷
G1-1624	fill	Late Neolithic	below plough soil, above G1-1627	1 plant	KFO6
G1-1627	destruction layer	Late Neolithic	pre-dates G1-1624	1 plant	KFO7
G1-2000	fill containing flecks of charcoal	transitional MN/LN, here treated as LN due to the appearance of Black Ware pottery (see text)	S	9 faunal	KOUP-57, KOUP-58, KOUP-59, KOUP-60, KFO61, KFO62, KFO63, KFO64, KFO65
G1-2001	fill containing flecks of charcoal (fewer in number than G1-2000)	transitional MN/LN, here treated as LN due to the appearance of Black Ware pottery (see text)	likely same horizon as G1- 1666	11 faunal	KOUP-64, KOUP-65, KOUP-66, KOUP-67, KOUP-68, KOUP69, KOUP-70, KOUP-71, KFO66, KFO67, KFO68
G1-1666	fill of a cut (likely hearth structure) containing charcoal and burnt animal bones	transitional MN/LN, here treated as LN due to the appearance of Black Ware pottery (see text); AMS date of hulled barley grains from this context: 5341-5213 cal BC	likely same horizon as G1- 2001 excavated in a different year	1 plant	KFO22
G1-2003	mixed dumped deposit containing charcoal flecks extending over entire G1 sounding	transitional MN/LN, here treated as LN due to the appearance of Black Ware pottery (see text); AMS date of hulled barley grains from this context: 5319-5209 cal BC	pre-dates G1-2001, G1- 2000 and G1-1666	1 plant	KFO20

Area G2					
G2-1103	first artificial spit of fill above destruction collapse extending over the entire G2 area	Late Neolithic	post-dates G2-1102	2 faunal	KOUP-79, KFO69
G2-1104	second artificial spit of fill above destruction collapse extending over the whole G2 area	Late Neolithic	post-dates G2-1102, same unit as G2-1103	2 faunal	KFO71, KOUP-81
G2-1102	collapsed wall from a working area	Late Neolithic	pre-dates G2-1103 and G2-1104	1 faunal	KOUP-75
G2-1115	fill below a working area	Late Neolithic	5	2 faunal	KOUP-83, KOUP-84
G2-1132	fill	Late Neolithic; AMS date of a hulled barley grain from this context: 5325-5207 cal BC	post-dates G2-1136	4 faunal	KFO72, KFO73, KFO74, KOUP-88
G2-1136	context containing no features and extending over the whole G2 area	transitional MN/LN, here treated as LN due to the appearance of Black Ware pottery (see text)	pre-dates G2-1132	4 faunal	KFO75, KFO76, KOUP-92, KOUP-93
G2-1139	fill extending over the whole G2 area containing a fair amount of charcoal	transitional MN/LN, here treated as LN due to the appearance of Black Ware pottery (see text); AMS date of peas from this context: 5468-5214 cal BC	pre-dates G2-1136	2 faunal	KOUP-96, KOUP-97
Area B					
B0134	oval shaped deposit with fragments of mud brick, bone, and chipped stone	Late Neolithic	pre-dates a LN horizon and post-dates some LN contexts	2 plant	KFO1, KFO21
B0185	clay deposit extending over much of the eastern sector of Area B	transitional MN/LN, here treated as LN due to the appearance of Black Ware pottery	re-dates B0134	1 plant	KFO2

		(see text)			
Area H				(
H0709	fill extending over the entire trench of Area H, containing numerous flecks of charcoal	Late Neolithic	pre-dates a LN horizon	2 plant	KFO8, KFO23

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		%C	%N	C:N	
Kouphovouno ce	reals (n=20)				
KFO2	KFO ft wheat	48.6	3.1	18.3	
KFO3	KFO ft wheat	46.9	2.8	19.6	
KFO5	KFO ft wheat	48.1	3.2	17.5	
KFO6	KFO ft wheat	56.1	3.1	21.1	
KFO7	KFO ft wheat	50.6	2.8	21.3	
KFO9	KFO ft wheat	49.0	3.1	18.3	
KFO10	KFO ft wheat	51.3	2.9	20.6	
KFO11	KFO ft wheat	40.1	3.4	13.6	
KFO12	KFO ft wheat	36.0	2.2	19.5	
KFO13	KFO ft wheat	51.5	2.9	20.4	
KFO16	KFO ft wheat	40.1	2.9	16.2	
KFO18	KFO ft wheat	46.2	2.8	19.2	
KFO19	KFO ft wheat	50.8	3.1	19.0	
KFO1	KFO h barley	43.4	2.0	25.7	
KFO4	KFO h barley	49.8	2.3	25.3	
KFO8	KFO h barley	56.0	1.6	40.3	
KFO14	KFO h barley	54.3	2.3	27.4	
KFO15	KFO h barley	50.2	2.0	29.1	
KFO17	KFO h barley	44.1	2.2	23.1	
KFO20	KFO h barley	45.1	2.0	26.1	
		SD	(1 σ) of C:N	5.8	
			C:N max	40.3	
Kaunhawauna nu	leas (n_7)		C:N min	13.6	
		46.6	4.2	10.6	
KFO21	KFO pea	40.0	4.3 5.2	12.0	
	KFO pea	20.4	0.0	67	
KFO23	KFO pea	18 2	0.0	0.7	
KFO24 KEO27	KFO pea	40.2	0.0	9.5	
KEO28	KFO pea	38.0	4.4	12.4	
KFO29	KFO nea	45.3	4.5	12.0	
11 025	ni o pea	40.0	4.0	11.0	
		SD	(1 σ) of C:N	2.3	
		_	C:N max	13.4	
			C:N min	6.7	
)				
Modern charred o	ereals (n=18)				
BAD04-18W	bread wheat	59.0	1.9	36.2	
BOR07-44W	bread wheat	62.3	2.8	26.3	
SUT08-37K	einkorn	66.1	3.7	20.8	
SUT08-31K	einkorn	63.2	3.8	19.5	
ASK08-513E	emmer	62.2	4.1	17.9	
ASK08-524E	emmer	60.7	2.9	24.4	
ASK08-526E	emmer	62.4	3.4	21.3	
ASK08-532E	emmer	62.0	3.2	22.6	
ASK08-544E	emmer	63.4	3.7	20.3	

SUT08-25E	emmer	67.1	3.3	24.1	
ASK08-513G	naked barley	63.8	3.4	21.8	
ASK08-524G	naked barley	64.1	3.9	19.2	
ASK08-526G	naked barley	63.6	3.6	20.6	
ASK08-532G	naked barley	63.0	3.9	18.8	
ASK08-544G	naked barley	64.5	3.2	23.3	
BAD07-6G	hulled barley	63.9	2.6	28.2	
BAD07-12G	hulled barley	63.3	3.3	22.6	
BAD07-18G	hulled barley	63.3	2.9	25.6	
		SD	(1 σ) of C:N	4.3	
			C:N max	36.2	
			C:N min	17.9	
Modern charred pulses (n=15)					
BAD07-6P	pea	64.9	7.1	10.7	
BAD08-6P	pea	65.0	6.0	12.7	
BAD08-12P	pea	65.5	5.8	13.1	
BAD08-18P	pea	65.6	6.3	12.2	
BAD07-18P	pea	58.1	6.5	10.5	
ICA09-11L	lentil	64.5	6.4	11.8	
ICA09-12L	lentil	64.1	7.0	10.7	
ICA09-13L	lentil	63.1	6.4	11.5	
ICA09-14L	lentil	62.7	7.1	10.4	
ICA09-15L	lentil	62.2	7.0	10.4	
ICA09-16L	lentil	64.1	6.8	11.1	
ICA09-17L	lentil	62.1	6.8	10.7	
BAD08-6B	broad bean	62.6	8.3	8.8	
BAD07-12B	broad bean	62.3	7.5	9.6	
EVV07-25B	broad bean	57.2	6.7	10.0	
		SD	(1 σ) of C:N	1.2	
			C:N max	13.1	
			C:N min	8.8	