

1 Further insight into Neolithic agricultural management at Kouphovouno,  
2 southern Greece: expanding the isotopic approach  
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4 Petra Vaiglova<sup>a,b,\*</sup>, Armelle Gardeisen<sup>c</sup>, Michael Buckley<sup>d</sup>, William Cavanagh<sup>e</sup>,  
5 Josette Renard<sup>f</sup>, Julia Lee-Thorp<sup>g</sup>, Amy Bogaard<sup>h</sup>  
6

7 <sup>a</sup> Department of Anthropology, Washington University in St Louis, 1 Brookings Drive, St Louis,  
8 Missouri 63130, USA

9 <sup>b</sup> Malcolm H. Wiener Laboratory for Archaeological Science, American School of Classical  
10 Studies at Athens, 54 Souidias street, Athens 106 76, Greece

11 <sup>c</sup> Centre National de la Recherche Scientifique/Unité Mixte de Recherche, Lattes 34970, France

12 <sup>d</sup> Manchester Institute of Biotechnology, Faculty of Life Sciences, 131 Princess Street, Manchester  
13 M1 7DN, UK

14 <sup>e</sup> Department of Archaeology, University of Nottingham, University Park, Nottingham NG7 2RD,  
15 UK

16 <sup>f</sup> Université Paul-Valéry, Montpellier 3, Route de Mende, F-34199 Montpellier, France

17 <sup>g</sup> Research Laboratory for Archaeology and the History of Art, University of Oxford, South  
18 Parks Road, Oxford OX1 3QY, United Kingdom

19 <sup>h</sup> Institute of Archaeology, University of Oxford, 36 Beaumont street, Oxford OX1 2PG, UK  
20  
21

22 \* corresponding author: vaiglova.petra@gmail.com, tel: (860) 486-2137, ORCID: 0000-0002-  
23 9468-8138  
24  
25

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## Abstract

This paper investigates agricultural management choices of farmers at the Neolithic site of Kouphvouno, southern Greece. Previous stable isotopic analysis of charred plant remains and bone animal collagen showed that throughout the Neolithic occupation of this site, farmers employed species-specific strategies to cultivate crops and herd domestic animals. Additional analyses of charred plant remains carried out in this study (including einkorn, a cereal species not measured before) expand our understanding of the diversity and flexibility of early crop cultivation on a local scale. Furthermore, sequential tooth enamel carbonate isotopic analyses are used to assess the seasonal dietary and grazing patterns of domestic sheep and goat, providing a more nuanced picture of the roles of these animals in the subsistence economy of this community. The results show that the species-specific cultivation system was dictated by the crops' ecological adaptations. Based on a small number of individuals available for analysis, the findings suggest that animal management was also likely driven by cultural choices, and involved foddering of goats managed for milk and local grazing of sheep managed for meat.

## Keywords

Isotopic analysis, archaeobotany, archaeozoology, Aegean prehistory, archaeological science, agriculture

## 1. Introduction

Applications of stable isotopic analysis in the field of Neolithic archaeology have made insightful contributions to our understanding of *how* early farming systems functioned, shedding light on the scale and intensity of ancient crop cultivation as well as seasonal and multi-annual patterns in livestock herding management (Balasse et al. 2012; Bogaard et al. 2013; Makarewicz et al. 2017). At the Middle–Late Neolithic site of Kouphovouno, southern Greece, an investigation that integrated stable isotopic measurements of archaeological plant and animal remains provided some of the first direct evidence for species-specific management strategies employed by Neolithic farmers (Vaiglova et al. 2014a). Furthermore, it presented preliminary insight into subtle differences between long-term average diets of domestic sheep and goats. These findings support

1 the model – based on results of systematic archaeobotanical and archaeozoological analyses  
2 (Halstead 1981, 2011; Bogaard 2004, 2005) – that the management techniques employed by these  
3 ancient agropastoralists were small-scale and intensive. The pilot study raised further questions  
4 regarding the differential management of crops and seasonal herding patterns of animals that  
5 formed the basis of this mixed farming economy.

6  
7 This paper presents the results of a second phase of crop and animal isotopic analyses carried out  
8 at Kouphovouno. Additional samples (including a crop species not measured before) became  
9 available with the completion of the archaeobotanical analysis, enabling a more thorough  
10 assessment of the crop cultivation practices developed by the Neolithic farmers. Furthermore,  
11 sequential analysis of tooth enamel carbonate of sheep and goats from both phases of the site's  
12 occupation was used to assess the animals' seasonal and multi-annual patterns in grazing behavior.  
13 The combined crop and animal stable isotopic results further our understanding of the symbiotic  
14 relationship between crop cultivation and animal husbandry in an early farming context. This  
15 information provides direct insight into the roles that plants and animals played in the local  
16 subsistence economy, as well as the ways that people adapted their management strategies to their  
17 environmental constraints and opportunities.

## 18 19 **2. The archaeological site of Kouphovouno**

20 Kouphovouno is a Middle–Late Neolithic (5800 – 5000 cal BC, Mee et al. 2014) site located about  
21 2.5 km southwest of modern-day Sparta, southern Greece (see Fig.1). The tell site rises to an  
22 elevation of 5 m above the surrounding Sparta basin and spreads over an area of 4–5 ha. The  
23 mound is bordered in the north by a seasonal stream, Parori, which originates in a system of alluvial  
24 fans to the west and feeds into the Evrotas river 3.5 km to the east (Cavanagh et al. 2004, 2007).  
25 To the south, the mound is met by a perennial pond (Fouache et al. 2007). The alluvial fans occupy  
26 the piedmont zone of the Taygetos Mountains, a limestone massif that reaches an elevation of  
27 2,404 masl. Geomorphological reconstruction suggests that in the Neolithic, water was abundant  
28 close to the surface of the Sparta basin, causing annual flooding (Fouache et al. 2007).

29  
30 The excavated portions of the Neolithic village indicate that the settlement was nucleated and may  
31 have been divided into neighborhoods. There were refuse disposal areas located in close proximity

1 to the habitation spaces. A possible garden was identified in Area G. No human remains dating to  
2 the Neolithic period were found (Cavanagh et al. 2004, 2007). The transition from Middle  
3 Neolithic (MN) to Late Neolithic (LN) (~ 5400 cal BC) was marked by a shift from more  
4 permanent to more ephemeral architecture, accompanied by a change from a more uniform  
5 ceramic assemblage to one that is more diverse and contains black ware pottery (Mee et al. 2014).  
6 The data from Kouphovouno suggests that the Middle to Late Neolithic transition in southern  
7 Greece was not as abrupt as previously thought (Mee et al. 2014). Cavanagh and Renard (2014)  
8 argue that the diverse collection of figurines, together with obsidian and flint blades, suggests that  
9 the Neolithic inhabitants participated in a network of exchange that extended across the Adriatic  
10 zone (the Balkans to the north and Italy to the west) and into the eastern Aegean.

11  
12 The plant assemblage from Kouphovouno is dominated by domestic crop species, mainly cereals:  
13 one-seeded einkorn wheat (*Triticum monococcum*), emmer wheat (*Triticum dicoccum*), free-  
14 threshing wheat (*Triticum aestivum/Triticum durum*), lentil (*Lens culinaris*), pea (*Pisum sativum*),  
15 and bitter vetch (*Vicia ervilia*). The high incidence of free-threshing wheat at Kouphovouno  
16 presents a contrast to the situation in northern Greece, where farmers are argued to have rejected  
17 this crop by choice (Valamoti and Jones 2003). At Kouphovouno, free-threshing wheat is not only  
18 found in rich storage contexts, but previous stable isotope analysis suggests that it was cultivated  
19 under more intensively maintained growing conditions (likely using the application of farmyard  
20 manure or other organic waste) than hulled barley. Furthermore, it may have been grown  
21 exclusively for human consumption (Vaiglova et al. 2014a). The stable isotopic values of einkorn  
22 were not previously measured, and the opportunity to do so in this study provides a chance to  
23 expand our understanding of crop cultivation systems employed by the Neolithic farmers at the  
24 site. The previous isotopic work also suggests that peas were grown at a high intensity, possibly  
25 in rotation with free-threshing wheat but under a higher water status than the cereals, likely using  
26 hand-watering.

27  
28 The faunal assemblage is dominated by domestic species, namely sheep (*Ovis aries*), goats (*Capra*  
29 sp.), pigs (*Sus scrofa*), and cattle (*Bos taurus*). Analysis of the mortality profiles of the ovicaprids  
30 (sheep and goats) suggests that either the farmers practiced a mixed primary and secondary  
31 products exploitation strategy during both periods of occupation or that the exploitation strategy

shifted focus from secondary products in the Middle Neolithic to meat in the Late Neolithic (Cantuel et al. 2008). The mortality profiles of the cattle could not be determined due to the low numbers of preserved individuals. Analysis of dental micro- and meso-wear patterns did not indicate any dietary differences between sheep and goats (distinguished using morphological criteria) in the last 2-3 weeks of their lives (Rivals et al. 2011). Stable isotope analysis of the animals' bone collagen, identified to species using Zooarchaeology Mass Spectrometry (ZooMS), however, suggests that there was a chronological shift in the long-term average diets of the ovicaprids, which points to a change in the management strategy over time (Vaiglova et al. 2014a).

### 3. Principles of stable isotope analysis

Isotopic ratios of organic (bone collagen, charred grains) and inorganic (tooth enamel) materials recovered from archaeological sites contain information about past climatic conditions, animal seasonal dietary behavior and plant growing conditions (Balasse et al. 1999; Bogaard et al. 2007; Lee-Thorp 2008). Stable carbon isotopic values ( $^{13}\text{C}/^{12}\text{C}$ ,  $\delta^{13}\text{C}$ ) of plants are mainly determined by their photosynthetic pathway ( $\text{C}_3$  or  $\text{C}_4$ ) and to a smaller degree by environmental factors including temperature, humidity, light and air pressure (Ehleringer et al. 1991; Tieszen 1991; Cerling et al. 1997; Hartman and Danin 2010).

$\text{C}_3$  plants include domestic crops such as *Triticum monococcum* and have  $\delta^{13}\text{C}$  values ranging from -34 to -21‰, with an average around -27‰.  $\text{C}_4$  plants include arid-adapted grasses such as *Cynodon dactylon* and have  $\delta^{13}\text{C}$  values ranging from -17 to -9‰, with an average around -12‰ (Vogel and van der Merwe 1977; Cerling et al. 1997). Plant  $\delta^{13}\text{C}$  values are negatively correlated with moisture availability, so plants growing in regions with higher mean annual rainfall have more negative  $\delta^{13}\text{C}$  values compared to plants growing in drier regions (Hartman and Danin 2010). Physiological differences in carbon assimilation of plants utilizing the same photosynthetic pathway also cause small differences in  $\delta^{13}\text{C}$  values (Hartman and Danin 2010). For example, barley has been found to have consistently lower  $\delta^{13}\text{C}$  values than wheat grown under the same moisture availability (Araus et al. 1997a, b). In Mediterranean climates, the  $\delta^{13}\text{C}$  values of plants are highest during the warm/dry summers due to increased evapotranspiration which causes  $^{13}\text{C}$  enrichment, and lowest during the cold/wet winters (Hartman and Danin 2010).

1 Stable nitrogen isotope values ( $^{15}\text{N}/^{14}\text{N}$ ,  $\delta^{15}\text{N}$ ) values of plants are primarily determined by the  
2 source from which the plants obtain nitrogen (Sharp 2007; Szpak 2014). Nitrogen-fixers (such as  
3 members of the *Leguminosae* family) host mycorrhizae fungi that fix  $\text{N}_2$  directly from the  
4 atmosphere. As a result, they have values close to 0‰, which is the  $\delta^{15}\text{N}$  value of AIR (atmospheric  
5 inhalable reservoir) (Craine et al. 2009; Szpak 2014). Nitrogen non-fixers (such as *Triticum* and  
6 *Hordeum*) assimilate nitrogen from soil and are thus influenced by a host of natural and  
7 anthropogenic soil  $^{15}\text{N}$  enrichment factors. Higher temperatures and lower precipitation create  
8 conditions that are more prone to N loss, and thus cause  $^{15}\text{N}$  enrichment (Szpak 2014). Mean  
9 annual precipitation is negatively correlated with plant  $\delta^{15}\text{N}$  values, so plants growing in more arid  
10 regions tend to have higher  $\delta^{15}\text{N}$  values compared to plants growing in wetter regions (Heaton et  
11 al. 1986; Ambrose 1991; Craine et al. 2009; Hartman and Danin 2010). However, certain chemical  
12 transformations that occur in soil, such as denitrification (which involves the transformation of  
13 inorganic nitrate,  $\text{NO}_3^-$ , into gaseous  $\text{N}_2$ ) also cause soil  $^{15}\text{N}$  enrichment (Tiedje et al. 1982;  
14 Högberg 1997), so an understanding of a site's hydrological history is crucial for the interpretation  
15 of past soil and plant  $\delta^{15}\text{N}$  values.

16  
17 Anthropogenic factors that drive soil  $^{15}\text{N}$  enrichment include methods to improve soil fertility such  
18 as tillage, burning and application of animal manure or decomposing midden material (Bateman  
19 et al. 2005; Bogaard et al. 2007; Fraser et al. 2011; Hobbie and Högberg 2012; Szpak 2014).  
20 Tillage promotes mineralization of soil organic matter and brings soil from deeper in a soil profile  
21 (and more enriched in  $^{15}\text{N}$ ) closer to the surface (Szpak 2014). Manure/compost undergo increased  
22 ammonia volatilization, which causes loss of the lighter  $^{14}\text{N}$  in the form of gaseous  $\text{NH}_3$ , leaving  
23 the substrate enriched in  $^{15}\text{N}$ . Plants that grow in heavily managed soil thus have higher  $\delta^{15}\text{N}$  values  
24 than plants growing in unmanaged soils.

25  
26 Animals assimilate C and N from food into their hard and soft tissues and their isotopic values  
27 become enriched at every level of the food chain. Thus, carnivores have higher  $\delta^{15}\text{N}$  values  
28 compared to herbivores, and herbivores have higher  $\delta^{15}\text{N}$  compared to the plants they consume  
29 (DeNiro and Epstein 1981; Minagawa and Wada 1984; Hedges and Reynard 2007). The diet to  
30 tissue offset is between 3–6‰ in  $\delta^{15}\text{N}$  values of bone collagen (Schoeninger and DeNiro 1984;  
31 Bocherens and Drucker 2003; O'Connell et al. 2012), 3–6‰ for  $\delta^{13}\text{C}$  in bone collagen and 9–15‰

1 for  $\delta^{13}\text{C}$  in enamel bioapatite; the actual offset depending on the animals' digestive physiology  
2 (van der Merwe and Vogel 1978; Krueger and Sullivan 1984; Lee-Thorp et al. 1989; Cerling and  
3 Harris 1999). Animals that consume mixed  $\text{C}_3$  and  $\text{C}_4$  diets have tooth enamel values above -8‰  
4 (Cerling et al. 1997). Animal bones remodel themselves throughout the life of individuals at  
5 varying rates (DeNiro and Epstein 1981). The isotopic composition of bone collagen, which is  
6 more diagenetically robust than the mineral portion of bone (Hare 1980; Collins et al. 2002), thus  
7 reflects the dietary inputs over the last several years of the individuals' lives. Carbon is  
8 preferentially routed from the protein component of diets and together,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of  
9 bone collagen from archaeological specimens serve as indicators of their long-term dietary protein  
10 intake (Krueger and Sullivan 1984; Ambrose and Norr 1993; Tieszen and Fagre 1993).

11  
12 Animals incorporate oxygen into hard tissues like tooth enamel mainly from ingested food and  
13 water, which in turn reflect the isotopic composition of meteoric, surface and plant leaf water  
14 (Longinelli 1984; Luz et al. 1984; Bryant et al. 1996). Stable oxygen isotopic values ( $^{18}\text{O}/^{16}\text{O}$ ,  
15  $\delta^{18}\text{O}$ ) of meteoric water are driven by fractionation during the hydrological cycle, so that  
16 precipitation closer to the coast is isotopically lighter compared to precipitation in upland areas  
17 (Dansgaard 1964; Bowen and Wilkinson 2002; Hoefs 2009). At mid to high latitudes, higher  
18 temperatures and increased rates of evapotranspiration favor  $^{18}\text{O}$  enrichment in surface and plant  
19 leaf water, resulting in higher  $\delta^{18}\text{O}$  values in the summer and lower values in the winter (Gat 1980;  
20 Allison and Hughes 1983; Rozanski et al. 1993).

21  
22 Enamel mineralizes sequentially along the axis of tooth growth (starting at the tooth crown and  
23 ending at the enamel root junction), incorporating oxygen from ingested water in equilibrium with  
24 body water (Land et al. 1980; Luz et al. 1984). The second molars (M2) of herbivores like sheep  
25 and goats complete mineralization during the first 12-13 months of the individuals' lives (Brown  
26 et al. 1960; Weinreb and Sharav 1964; Balasse et al. 2001; Hillson 2005; Towers et al. 2014). Once  
27 mineralized during the developmental years, tooth enamel does not remodel itself, and its isotopic  
28 composition thus reflects the dietary and water inputs consumed during the mineralization period.  
29  $\delta^{18}\text{O}$  values of enamel samples taken along the axis of tooth growth reflect an entire annual cycle  
30 of seasonal changes in  $\delta^{18}\text{O}$  values. Sequential enamel  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  values thus provide  
31 information on the seasonal changes in the animals' dietary and mobility patterns and serve as

1 short-term indicators of their grazing behavior (Bocherens et al. 2001; Balasse 2002; Balasse et al.  
2 2002).

3  
4 Individuals that eat fresh vegetation and do not migrate across altitudes during the tooth  
5 mineralization period typically have sinusoidal intra-tooth  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  value sequences, with  
6 the maximum values reflecting composition of plants and water ingested during the summer and  
7 minimum values reflecting the composition of plants and water ingested during the winter (Balasse  
8 et al. 2002, 2013; Kirsanow et al. 2008). Animals that eat collected fodder for part of the year tend  
9 to have flattened  $\delta^{13}\text{C}$  value sequences, since the consumed vegetation does not reflect the seasonal  
10 changes in carbon isotopic discrimination (Makarewicz 2014). However, if they are obligate  
11 drinkers (like sheep and goats), their  $\delta^{18}\text{O}$  sequences will still fluctuate predictably due to the  
12 seasonal changes in temperature and evapotranspiration. Animals that migrate across altitudinal  
13 boundaries, such as those that partake in transhumant pastoralism and spend the dry season at  
14 higher altitudes, can show dampened  $\delta^{18}\text{O}$  value sequences due to consumption of water that does  
15 not reflect the annual extremes (Longinelli and Selmo 2003; Britton et al. 2009; Henton et al.  
16 2010).

#### 18 **4. Materials and methods**

19 In the first part of this study, we measured the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of samples from the  
20 archaeobotanical assemblage that were not available during the pilot study. In the second part, we  
21 obtained incremental samples of tooth enamel carbonate of sheep and goats, and measured the  
22 seasonal fluctuations in  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values. Permission to carry out the scientific analyses was  
23 granted by the excavation directors (Prof William Cavanagh, Prof Christopher Mee, Prof Josette  
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##### 27 **4.1. Stage 1: Additional charred plant isotope analysis**

28  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were measured from 19 samples of charred grains/seeds (see Table 1). Each  
29 sample was taken from a discrete contextual unit and included between 3 and 13 whole  
30 grains/seeds, or fragments thereof. The chronological assignments were made on the basis of  
31 stratigraphic associations and radiocarbon dating. Five contexts analyzed in this study and by



Vaiglova et al. (2014a) (C0854, C0848, C0844, G2003, G1139) have been directly dated using radiocarbon (Mee et al. 2014). Only samples that were charred in the ‘optimal charring window’ (i.e., at charring conditions that do not obscure the original isotopic signatures, cf Nitsch et al. 2015) were measured. All samples were chemically pre-treated using a gentle acid-only treatment with 0.5 M HCl at 80°C for 30min (Vaiglova et al. 2014b).

**Table 1** Charred plant samples from Kouphovouno analyzed in stage 1 of this study

Crop category	Common name	Latin name	n =
Cereal	einkorn <sup>a</sup>	<i>Triticum monococcum</i> L.	6
	free-threshing wheat	<i>Triticum aestivum</i> Desf. / <i>Triticum durum</i> Desf.	4
	hulled barley	<i>Hordeum vulgare</i> L.	8
Pulse	grass pea	<i>Lathyrus sativus</i> L.	1

<sup>a</sup> all samples of einkorn belonged to the one-grained variety

Stable carbon and nitrogen isotopic compositions were determined using a SerCon 20/22 continuous flow mass spectrometer coupled to a Callisto elemental analyzer at the Research Laboratory for Archaeology and the History of Art, University of Oxford. Analysis was carried out separately for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, as the target weights differed for the two sets of measurements.  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were calibrated relative to VPDB and AIR, respectively. Measurement uncertainty for  $\delta^{13}\text{C}$  was monitored using international reference standards: CH-6 ( $\delta^{13}\text{C} = -10.45 \pm 0.03 \text{ ‰}$ ) and CH-7 ( $\delta^{13}\text{C} = -32.15 \pm 0.05 \text{ ‰}$ ). Measurement uncertainty for  $\delta^{15}\text{N}$  was monitored using international reference standards N2 ( $\delta^{15}\text{N} = 20.3 \pm 0.02 \text{ ‰}$ ) and caffeine ( $\delta^{15}\text{N} = 2.9 \pm 0.03 \text{ ‰}$ ). Precision ( $u(R_w)$ ) was determined to be  $\pm 0.48$  for  $\delta^{13}\text{C}$  and  $\pm 0.29$  for  $\delta^{15}\text{N}$  on the basis of repeated measurements of calibration standards and check standards. Accuracy ( $u(\text{bias})$ ) was determined to be  $\pm 0.38$  for  $\delta^{13}\text{C}$  and  $0.34$  for  $\delta^{15}\text{N}$  on the basis of the difference between the observed and known  $\delta$  values of the check standards and the long-term standard deviations of the check standards. Using the equations from Szpak et al. (2017), the total analytical uncertainty was estimated to be  $\pm 0.62$  for  $\delta^{13}\text{C}$  and  $\pm 0.45$  for  $\delta^{15}\text{N}$ .

All plant isotope values were corrected for an average charring-induced isotopic enrichment of  $0.3\text{‰}$  in  $\delta^{15}\text{N}$  values and  $0.1\text{‰}$  in  $\delta^{13}\text{C}$  values (the confidence intervals for these average values

are 0.003-0.22‰ for  $\delta^{13}\text{C}$  and 0.05-0.57‰ for  $\delta^{15}\text{N}$  (Nitsch et al. 2015). The previously measured plant data from this assemblage (n=28) were corrected using the more recent charring offset (the offset at the time of the first publication was 1.0‰ in  $\delta^{15}\text{N}$ , cf Fraser et al. 2013) and will be presented here alongside the new measurements. This new charring correction did not change the interpretation of the previous results, as the distinction between the free-threshing wheat  $\delta^{15}\text{N}$  values and the hulled barley  $\delta^{15}\text{N}$  values remains the same (even though all the absolute corrected values are 0.7‰ higher).

The  $\Delta^{13}\text{C}_{\text{air}}$  for the Neolithic period was estimated to be -6.5‰ using the AIRCO2\_LOESS data calibrator (Cleveland 1979; Leuenberger et al. 1992; Indermühle et al. 1999; Ferrio et al. 2005). One sample of hulled barley (KFO34) and one sample of free-threshing wheat (KFO50), both from the MN, only yielded reliable  $\delta^{15}\text{N}$  measurements. One sample of free-threshing wheat (KFO52) only yielded a reliable  $\delta^{13}\text{C}$  measurement.

#### **4.2. Stage 2: Tooth enamel carbonate analysis**

Sequential samples of tooth enamel were obtained from the second molars (M2) of sheep (n=4) and goats (n=4) from Kouphovouno. M2s mineralize within 12-13 months of the animals' birth (Brown et al. 1960; Balasse et al. 2001). Because enamel does not remodel once mineralized, the isotopic values of M2s thus reflect the dietary and water inputs during the first year of the animals' lives. Three sheep (KFO209, KFO217, KFO210) and two goats (KFO204, KFO207) date to the Middle Neolithic and one sheep (KFO212) and two goats (KFO214, KFO216) date to the Late Neolithic. The chronological assignments were made on the basis of stratigraphic associations and radiocarbon dating (Mee et al. 2014). Zooarchaeology Mass Spectrometry (ZooMS) (Buckley et al. 2010), carried out at the University of Manchester, was used to confirm the species identification of the samples. 6 out of the 8 samples were extracted from fully preserved mandibles, and these were aged using tooth wear stages established by Payne (1973). However, due to the state of preservation of the teeth, most of these identifications are uncertain (see Table 2).

Prior to sampling, the external surface of the teeth was cleaned using a laboratory aluminium oxide sandblaster and a Dremel tool with a tungsten drill bit. 5–10 mg sub-samples of powdered enamel were removed at 1 mm intervals along the axis of growth from the buccal side of each tooth,

1 starting at the occlusal surface and ending at the enamel root junction (erj), following the protocol  
2 established by Balasse (2002) (see Fig.2 for an image of a sampled tooth). The number of sub-  
3 samples per tooth ranged from 15–16 in sheep (total n=61) and 11–22 in goats (total n=65). The  
4 crown lengths ranged from 22–30 mm in sheep and 24–30 mm in goats.

5  
6 All samples were pre-treated using 1 M Ca-buffered acetic acid for 30 min at room temperature in  
7 order to remove possible exogenous carbonate contamination (Snoeck and Pellegrini 2015). Stable  
8 carbon and oxygen isotopic compositions were determined using a Thermo Gas Bench II device  
9 coupled to a Thermo Delta V Advantage mass spectrometer at the School of Archaeological  
10 Sciences, University of Bradford.  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values were calibrated relative to VPDB.  
11 Measurement uncertainty was monitored using international reference standards: CO-1 ( $\delta^{13}\text{C} =$   
12  $2.49 \pm 0.03 \text{ ‰}$ ,  $\delta^{18}\text{O} = 2.40 \pm 0.1 \text{ ‰}$ ), CO-8 ( $\delta^{13}\text{C} = -5.76 \pm 0.03 \text{ ‰}$ ,  $\delta^{18}\text{O} = -22.7 \pm 0.2 \text{ ‰}$ ) and  
13 NBS-19 ( $\delta^{13}\text{C} = 1.95 \text{ ‰}$ ,  $\delta^{18}\text{O} = -2.20$ ). Precision ( $u(R_w)$ ) was determined to be  $\pm 0.33$  for  $\delta^{13}\text{C}$  and  
14  $0.43$  for  $\delta^{18}\text{O}$  on the basis of repeated measurements of calibration standards and check standards.  
15 Accuracy ( $u(bias)$ ) was determined to be  $\pm 0.21$  for  $\delta^{13}\text{C}$  and  $0.4$  for  $\delta^{18}\text{O}$  on the basis of the  
16 difference between the observed and known  $\delta$  values of the check standards and the long-term  
17 standard deviations of the check standards. Using the equations from Szpak et al. (2017), the total  
18 analytical uncertainty was estimated to be  $\pm 0.39$  for  $\delta^{13}\text{C}$  and  $\pm 0.43$  for  $\delta^{18}\text{O}$ .

19  
20 All graphs presented in this paper were prepared using the software R (version 3.5.1).

**Table 2** Sheep and goat teeth from Kouphovouno analyzed in stage 2 of this study

Tooth ID	Species <sup>a</sup>	Context	Context type	Phase	Number of sub-samples	Wear stage <sup>b</sup>	Estimated age <sup>c</sup>	Crown height (in mm)	Loose tooth/from mandible
KFO209	sheep	C 1708/32	room fill	Middle Neolithic	15	?F-G	(3-6 years)	28	mandible
KFO210	sheep	C 1040/10	room fill	Late Neolithic	15	G	4-6 years	22	mandible
KFO212	sheep	G2 1136	accumulation of debris	Late Neolithic	15	?D	(1-2 years)	30	mandible
KFO217	sheep	C 1752	room floor	Middle Neolithic	16	E?	(2-3 years)	30	mandible
KFO204	goat	C 1731/4	midden fill	Middle Neolithic	11	G	4-6 years	24	mandible
KFO207	goat	C 1713	midden fill	Middle Neolithic	20	-		30	loose tooth
KFO214	goat	D 0308/18	accumulation of debris	Late Neolithic	16	E	2-3 years	28	mandible
KFO216	goat	G 1648	accumulation of debris	Late Neolithic	18	-		28	loose tooth

<sup>a</sup> Identified using ZooMS (see Materials and Methods)

<sup>b</sup> Wear stages identified following Payne (1973)

<sup>c</sup> parentheses indicate uncertainty in classification

## 5. Results

### 5.1. Stage 1: Additional plant $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values

Fig.3 shows all plant and animal  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values from Kouphovouno, including previously published plant ( $n=28$ ) and animal ( $n=68$ ) samples (Vaiglova et al. 2014a). Table 3 presents the new raw data and Table 4 shows the summary statistics of the combined plant datasets. This new set of analyses revealed an even larger variability in cereal  $\delta^{15}\text{N}$  values than seen previously ( $\delta^{15}\text{N}$  range: 6.2‰ for free-threshing wheat,  $n=17$ ; 7.3‰ for barley,  $n=15$ ; 6.0‰ for einkorn,  $n=6$ , see Fig.4). When an einkorn outlier (KFO35), which lies in the middle of the sheep/goat cluster, is removed, the  $\delta^{15}\text{N}$  value range of einkorn is reduced to 2.6‰. With reported  $\delta^{15}\text{N}$  values below 0‰, this dataset provides some of the lowest plant  $\delta^{15}\text{N}$  values measured from any archaeological site to date. Only one additional sample of a pulse crop (grass pea) was available for analysis, which provided a significantly lower  $\delta^{15}\text{N}$  value (-2.5‰) compared to the 7 pea samples measured previously.

Multivariate statistics were used to test the difference between the mean  $\delta^{15}\text{N}$  values of the species where  $n>1$  (free-threshing wheat, hulled barley, einkorn, pea). The einkorn outlier (KFO35) was not included in the analysis. The data are normally distributed (Shapiro-Wilk test,  $W=0.95$ ,  $p=0.06$ ), but not homogenous (Levene's test,  $F(3,39)=3.02$ ,  $p=0.041$ ), so the non-parametric Kruskal-Wallis test was used. The results show that there are significant differences between the mean  $\delta^{15}\text{N}$  values of the four groups ( $H(3)=27.21$ ,  $p<0.01$ ) and a post-hoc Bonferroni test reveals that the differences are between the free-threshing wheat and each of the other three species ( $p < 0.01$  in all pairs), but not between the pairs of the other species (hulled barley and einkorn,  $p=0.14$ , einkorn and pea,  $p=0.13$ , hulled barley and pea,  $p=1.0$ ).

**Table 3** Results of stable carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) isotopic values of charred plants from Kouphovouno obtained in stage 1 of this study (in ‰). All measurements were corrected for a charring-induced offset of 0.1‰ (for  $\delta^{13}\text{C}$  values) and 0.3‰ (for  $\delta^{15}\text{N}$  values) (cf Nitsch et al. 2015). SD denotes standard deviation

Sample ID	Species	Context	Area	Phase	$\delta^{13}\text{C}_{\text{VPDB}}$	$\delta^{13}\text{C}_{\text{corr.}}$	$\delta^{13}\text{C}$ SD	$\Delta^{13}\text{C}$	%C	$\delta^{15}\text{N}_{\text{AIR}}$	$\delta^{15}\text{N}_{\text{corr.}}$	$\delta^{15}\text{N}$ SD	%N	C:N
KFO30	hulled barley	G2001	G	LN	-22.5	-22.6	0.5	16.5	65.6	2.0	1.7	0.3	2.5	30.1
KFO31	einkorn	G1665	G	LN	-24.3	-24.4	0.9	18.3	62.5	1.5	1.2	0.4	3.6	20.0
KFO32	einkorn	G2001	G	LN	-24.2	-24.3	0.5	18.2	63.7	-0.1	-0.4	0.4	3.7	19.9
KFO33	hulled barley	B0185	B	LN	-23.4	-23.5	0.5	17.4	60.8	0.6	0.3	0.3	2.8	25.4
KFO34	hulled barley	C0918	C	MN						2.2	1.9	0.3	2.7	
KFO35	einkorn	C0825	C	MN	-20.1	-20.2	0.6	13.9	62.7	4.9	4.6	0.3	3.7	19.7
KFO36	einkorn	G2003	G	LN	-22.8	-22.9	0.5	16.8	63.9	-1.0	-1.3	0.4	3.1	24.2
KFO37	hulled barley	G1136	G	LN	-24.3	-24.4	0.5	18.3	54.1	0.6	0.3	0.3	2.4	26.8
KFO38	grass pea	G1136	G	LN	-25.0	-25.1	0.5	19.1	56.3	-2.2	-2.5	0.4	5.6	11.7
KFO39	free-threshing wheat	B0180	B	MN	-22.0	-22.1	0.5	16.0	60.7	2.8	2.5	0.3	3.6	19.7
KFO40	hulled barley	B0149	B	LN	-24.5	-24.6	0.5	18.5	65.2	-2.1	-2.4	0.4	2.2	34.9
KFO41	einkorn	G1136	G	LN	-23.8	-23.9	0.1	17.8	63.9	-1.0	-1.3	0.4	4.5	16.5
KFO42	hulled barley	C0848	C	MN	-24.2	-24.3	0.5	18.2	59.1	0.4	0.1	0.3	2.3	30.4
KFO43	einkorn	G1139	G	LN	-22.4	-22.5	0.5	16.4	63.7	0.8	0.5	0.3	3.3	22.8
KFO50	free-threshing wheat	C0266	C	MN						4.4	4.1	0.3	2.7	
KFO51	hulled barley	C0266	C	MN	-24.6	-24.7	0.5	18.7	45.8	0.5	0.2	0.3	2.2	24.7
KFO52	free-threshing wheat	C0295	C	MN	-22.9	-23.0	0.5	16.8	23.8					
KFO53	hulled barley	C0905	C	MN	-23.7	-23.8	0.5	17.8	47.9	0.5	0.2	0.3	2.4	23.0
KFO54	free-threshing wheat	C0905	C	MN	-21.6	-21.7	0.5	15.6	41.6	1.9	1.6	0.3	2.9	16.8

**Table 4** Summary statistics of all plant  $\Delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values from Kouphovouno (in ‰). The dataset includes samples measured previously (n = 28, Vaiglova et al. 2014a) and corrected using the more recent charring correction (see Materials and Methods). SD denotes standard deviation

Species	n=	$\delta^{15}\text{N}_{\text{max}}$	$\delta^{15}\text{N}_{\text{min}}$	$\delta^{15}\text{N}_{\text{mean}}$	$\delta^{15}\text{N}$ SD ( $1\sigma$ )	$\delta^{15}\text{N}_{\text{range}}$	$\Delta^{13}\text{C}_{\text{max}}$	$\Delta^{13}\text{C}_{\text{min}}$	$\Delta^{13}\text{C}_{\text{mean}}$	$\Delta^{13}\text{C}$ SD ( $1\sigma$ )	$\Delta^{13}\text{C}_{\text{range}}$
free-threshing wheat	17	7.8	1.6	5.8	1.7	6.2	17.4	15.6	16.6	0.5	1.9
hulled barley	15	4.9	-2.4	1.7	2.0	7.3	19.2	16.5	18.3	0.7	2.7
einkorn	6	4.6	-1.4	0.5	2.2	6.0	18.3	13.9	16.9	1.6	4.4
pea	7	2.4	1.6	2.0	0.3	0.8	20.2	17.4	19.0	1.0	2.8
grass pea	1	-2.5	-2.5	-	-	-	19.1	19.1	19.1	-	-
lentil	1	-	-	-	-	-	19.3	19.3	19.3	-	-

To calculate a measure of plant carbon discrimination irrespective of the concentration of atmospheric CO<sub>2</sub>, all  $\delta^{13}\text{C}$  values of all plant measurements were converted to  $\Delta^{13}\text{C}$  using the following equation (Farquhar et al. 1989):

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

The ranges in  $\Delta^{13}\text{C}$  values of the crops also increased with the additional measurements: 1.9‰ for free-threshing wheat, 2.7‰ for hulled barley, 4.4‰ for einkorn (see Fig.5 and Table 4). The data for the four main species (where  $n < 1$ ) are normally distributed (Shapiro-Wilk test,  $W=0.95$ ,  $p=0.09$ ) and homogenous (Levene's test,  $F(3,38)=1.12$ ,  $p=0.35$ ), so an ANOVA test was used to assess statistically significant differences between the mean  $\delta^{13}\text{C}$  values of the four groups. The results indicate that there are significant differences ( $F(3,38)=25.26$ ,  $p<0.01$ ) and a post-hoc Bonferroni test reveals that the differences are between free-threshing wheat and hulled barley ( $p<0.01$ ), free-threshing wheat and pea ( $p<0.01$ ), and einkorn and pea ( $p=0.004$ ).

Fig.6 shows all plant and animal  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values divided by chronological phase (free threshing wheat: MN=14, LN=3; hulled barley: MN=8, LN=7; einkorn: MN=1 (outlier), LN=5; pea: MN=4, LN=3). Barley is the only species represented equally in the two phases. The mean  $\delta^{13}\text{C}$  values of barley in the two periods are comparable, but the range is narrower in the Middle Neolithic ( $-24.4 \pm 0.3\text{‰}$ ) compared to the Late Neolithic ( $-24.2 \pm 0.9\text{‰}$ ). The  $\delta^{15}\text{N}$  values are similarly variable, but are overall higher in the MN ( $2.3 \pm 2.0\text{‰}$ ) compared to the LN ( $1.0 \pm 1.8\text{‰}$ ). A student's t-test showed that there is no statistically significant difference between the mean  $\delta^{15}\text{N}$  values of MN and LN barley ( $t=-1.35$ ,  $p=0.02$ ).

## 5.2. Stage 2: Tooth enamel carbonate $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values

Fig.7 shows the sequential  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values of sheep and goat teeth from Kouphovouno. Table 5 lists the individual measurements and Table 6 presents the summary statistics.  $\delta^{13}\text{C}$  values vary from -13.7 to -9‰ in sheep and -13.3 to -11.4‰ in goats, with mean intra-tooth variation of 3.2 (from 1.1 to 4.6‰) in sheep and 1.1‰ (from 0.4 to 1.6‰) in goats.  $\delta^{18}\text{O}$  values vary from -5.3 to



+1.2‰ in sheep and -4.0 to +2.2‰ in goats, with mean intra-tooth variation of 3.7‰ (from 2.6 to 5.0‰) in sheep and 4.5‰ (from 3.1 to 5.3‰) in goats.

None of the animals exhibit notable consumption of C<sub>4</sub> vegetation during the first year of their lives, as their  $\delta^{13}\text{C}$  values lie below the -8‰ threshold for a mixed C<sub>3</sub>-C<sub>4</sub> diet (cf Cerling et al. 1997) (see Fig.8 with carbon sequences only). Three out of the four sheep exhibit sinusoidal intra-tooth carbon and oxygen isotopic sequences that reflect seasonal fluctuation in moisture and temperature, recording higher values in the summer and lower values in the winter (cf Gat 1980; Allison and Hughes 1983; Rozanski et al. 1993). The intra-tooth  $\delta^{13}\text{C}$ -value sequences of all four goats and one of the sheep (from the MN) are nearly flat. One sheep (KFO212 from the LN) records the highest  $\delta^{13}\text{C}$  values of all the individuals, but these values are still below the -8‰ cut-off for C<sub>4</sub> input in mixed diets (cf Cerling et al. 1997). This sheep also has the flattest intra-tooth  $\delta^{18}\text{O}$ -value sequence of all the animals measured: it has an amplitude ( $\Delta^{18}\text{O}$  value) of 2.6‰, while all the other individuals have  $\Delta^{18}\text{O}$  values between 3.1 – 5.0‰ (see Fig.9 with oxygen sequences only).

**Table 5** Results of stable carbon ( $\delta^{13}\text{C}_{\text{VPDB}}$ ) and oxygen ( $\delta^{18}\text{O}_{\text{VPDB}}$ ) isotopic values of ovicaprid second molars from Kouphouvouno obtained in stage 2 of this study (in ‰). MN = Middle Neolithic. LN = Late Neolithic.  $\Delta$  values denote intra-tooth amplitude (difference between maximum and minimum values)

KFO204 (MN goat)		KFO207 (MN goat)		KFO209 (MN sheep)		KFO210 (LN sheep)		KFO212 (LN sheep)		KFO214 (LN goat)		KFO216 (LN goat)		KFO217 (MN sheep)	
$\delta^{13}\text{C}$	$\delta^{18}\text{O}$	$\delta^{13}\text{C}$	$\delta^{18}\text{O}$	$\delta^{13}\text{C}$	$\delta^{18}\text{O}$	$\delta^{13}\text{C}$	$\delta^{18}\text{O}$	$\delta^{13}\text{C}$	$\delta^{18}\text{O}$	$\delta^{13}\text{C}$	$\delta^{18}\text{O}$	$\delta^{13}\text{C}$	$\delta^{18}\text{O}$	$\delta^{13}\text{C}$	$\delta^{18}\text{O}$
-12.2	-2.9	-11.7	-1.5	-10.2	-2.8	-12.3	-1.5	-9.0	-1.4	-12.9	-2.9	-12.5	-3.7	-11.1	-4.2
-12.5	-1.1	-12.0	-1.2	-10.0	-2.9	-12.2	-2.2	-9.0	-2.0	-12.7	-3.8	-12.7	-3.5	-11.9	-4.6
-12.4	-0.4	-12.3	-2.2	-10.6	-3.7	-12.5	-1.7	-9.0	-1.8	-12.9	-2.5	-12.7	-3.2	-12.5	-4.3
-12.5	-0.2	-12.4	-2.9	-10.9	-3.9	-12.5	-0.9	-9.5	-2.1	-12.7	-2.0	-12.5	-2.9	-12.8	-4.2
-12.6	-0.3	-12.9	-2.3	-11.0	-4.4	-12.5	-1.6	-10.1	-2.3	-13.0	-1.1	-12.2	-2.0	-13.2	-3.9
-12.3	-0.9	-12.9	-2.8	-11.6	-4.2	-12.4	-1.5	-10.9	-2.5	-13.0	-0.8	-11.9	-0.8	-13.3	-3.5
-12.4	-0.6	-13.1	-2.2	-11.9	-5.0	-12.5	-0.7	-11.6	-3.0	-12.9	-0.1	-12.0	-0.3	-13.7	-3.2
-12.2	-2.3	-13.1	-2.3	-12.5	-5.3	-12.3	-1.6	-12.4	-3.5	-12.6	0.6	-11.9	-0.7	-13.5	-2.4
-12.5	-1.8	-13.3	-3.0	-12.9	-5.1	-12.4	0.1	-13.1	-3.9	-12.4	0.8	-11.8	0.7	-13.3	-1.2
-12.4	-2.7	-12.7	-2.0	-13.3	-5.2	-12.1	-0.1	-13.3	-4.0	-12.3	0.9	-11.5	0.7	-13.1	-0.4
-12.5	-3.3	-12.9	-1.4	-13.5	-4.8	-12.4	-0.1	-13.5	-4.0	-12.1	0.7	-11.6	-0.1	-12.3	0.4
$\Delta=0.4$	$\Delta=3.1$	-12.8	-0.8	-13.3	-3.8	-12.3	0.9	-13.1	-3.8	-12.2	-0.3	-11.7	-0.2	-11.2	-0.3
		-12.7	-0.4	-13.1	-2.5	-12.0	1.2	-12.2	-3.7	-12.3	-1.1	-11.4	-0.7	-10.5	-1.5
		-12.7	-0.2	-11.8	-1.7	-11.5	-0.3	-11.2	-2.6	-12.1	-1.6	-11.5	-1.2	-10.3	-3.5
		-12.1	0.8	-11.6	-1.5	-11.4	0.0	-10.6	-2.9	-12.3	-3.1	-11.4	-2.7	-10.8	-2.3
		-12.3	1.2	-10.6	-1.4	$\Delta=1.1$	$\Delta=3.3$	$\Delta=4.6$	$\Delta=2.6$	-12.2	-2.7	-11.7	-4.0	-12.6	-3.4
		-12.2	1.9	$\Delta=3.5$	$\Delta=3.8$					$\Delta=1.0$	$\Delta=4.7$	-12.2	-2.6	$\Delta=3.5$	$\Delta=5.0$
		-11.6	2.2									-12.7	-3.1		
		-12.2	1.4									$\Delta=1.3$	$\Delta=4.8$		
		-12.4	-0.4												
		$\Delta=1.6$	$\Delta=5.3$												

**Table 6** Summary statistics of sequential ovicaprid  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values from Kouphovouno (in ‰).  $\Delta$  values intra-tooth amplitude (difference between maximum and minimum values)

Tooth ID	$\delta^{13}\text{C}_{\text{max}}$	$\delta^{13}\text{C}_{\text{min}}$	$\Delta^{13}\text{C}$	$\delta^{18}\text{O}_{\text{max}}$	$\delta^{18}\text{O}_{\text{min}}$	$\Delta^{18}\text{O}$
sheep						
KFO209	-10.0	-13.5	3.5	-1.4	-5.3	3.8
KFO210	-11.4	-12.5	1.1	1.2	-2.2	3.3
KFO212	-9.0	-13.5	4.6	-1.4	-4.0	2.6
KFO217	-10.3	-13.7	3.5	0.4	-4.6	5.0
goat						
KFO204	-12.2	-12.6	0.4	-0.2	-3.3	3.1
KFO207	-11.6	-13.3	1.6	2.2	-3.0	5.3
KFO214	-12.1	-13.0	1.0	0.9	-3.8	4.7
KFO216	-11.4	-12.7	1.3	0.7	-4.0	4.8

## 6. Discussion

Previous stable isotopic analysis of plant and animal remains from Neolithic Kouphovouno indicated that farmers used distinct strategies for cultivating different species of cereals, and that they did not herd ovicaprids (sheep and goats) together (Vaiglova et al. 2014a). The results raised more questions about the dynamics of a small-scale intensive management system, and these questions formed the basis of additional stable isotopic work carried out in this study. The following discussion will:

- (a) draw on a larger plant stable isotopic dataset to expand our understanding of the range of cultivation regimes employed by the ancient farmers, and
- (b) paint a more nuanced picture of the seasonal and multi-annual patterns of caprine grazing behavior.

Together, these narratives will further our understanding of the way Neolithic farmers at Kouphovouno made use of their environmental resources and adapted their food production systems to their culinary preferences and socio-economic choices.

### 6.1. Crop management

Based on a sample size of 13 free-threshing wheat and 7 hulled barley bulk charred grain samples, previous measurements of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values showed that throughout the Middle to Late Neolithic, these two cereals were not cultivated in the same soil conditions. Free-threshing wheat was consistently sown in more  $^{15}\text{N}$ -enriched soils. Because this distinction was species-specific

1 rather than eco-systemic, it was argued that anthropogenic rather than natural factors were  
2 responsible for the differential soil enrichment (Vaiglova et al. 2014a). Notably, this distinction in  
3  $\delta^{15}\text{N}$  values exists for free-threshing wheat and hulled barley samples that come from the same  
4 archaeological contexts, suggesting that the higher  $\delta^{15}\text{N}$  values were not caused by localized soil-  
5 diagenetic factors.

6  
7 The most likely anthropogenic cause of soil enrichment was more intensive soil management,  
8 including application of farmyard manure, midden material, or other decomposing organic  
9 material, and tillage. The previous results suggest that while free-threshing wheat was cultivated  
10 using more intensive treatments, the soils in which barley grew may also have been  
11 manured/managed, but to a lesser extent than free-threshing wheat. As the  $\delta^{15}\text{N}$  values of the  
12 animals do not lie a trophic level above the values of the  $\delta^{15}\text{N}$  values of free-threshing wheat grain,  
13 it was argued that this crop was grown exclusively for human consumption. This left open the  
14 possibility that barley was cultivated (at least partially) as a fodder crop.

15  
16 The expanded dataset provides further support for distinct treatment of free-threshing wheat and  
17 barley, but it reduces the uniformity of the soil conditions in which the two crops grew. In the  
18 original dataset, the range of  $\Delta^{13}\text{C}$  values of barley was narrow (1.2‰), and this was taken to  
19 suggest that this crop was grown under restricted water availability. With the addition of 8 new  
20 barley samples, the range of  $\Delta^{13}\text{C}$  values increased to 2.6‰; the original indication of restricted  
21 soil wetness was thus a construct of the smaller sample size. Barley that was grown in rainfed  
22 conditions at modern-day organic farms in Morocco exhibited within-field ranges of values  
23 between 0.5‰ (Bellota farm) to 5.0‰ (Agda farm) (both farms receive 703 mm of rainfall per  
24 year) (Styring et al. 2016). This suggests that  $\Delta^{13}\text{C}$  values of crops grown under uniform  
25 environmental conditions can be variable, and that ranges below 2‰ should not necessarily be  
26 interpreted as indicative of more restricted water availability. Araus et al. (1997a, b) argue that  
27 there are physiological differences in  $^{13}\text{C}$  assimilation between wheat and barley, accounting for  
28 differences of 1–2‰ (with barley having higher  $\Delta^{13}\text{C}$  values than wheat grown under the same  
29 moisture availability). At Kouphovouno, the mean  $\Delta^{13}\text{C}$  values of barley ( $18.3 \pm 0.7\text{‰}$ ) are 1.7‰  
30 lower compared to the mean  $\delta^{13}\text{C}$  values of free-threshing wheat ( $16.6 \pm 0.5\text{‰}$ ), suggesting that  
31 the two crops were grown under similar moisture availability, likely in rain-fed conditions.

1  
2 The range of  $\delta^{15}\text{N}$  values of barley also increased with the addition of new samples, but none of  
3 the samples lie in the cluster where the majority of the free-threshing wheat sample lie (above  
4  $+4.9\text{‰}$ ). While the difference between the  $\delta^{15}\text{N}$  values of free-threshing wheat and barley remains  
5 statistically significant, the new data provides further evidence that that free-threshing wheat was  
6 cultivated in more intensively managed soils. Similar higher-intensity management of free-  
7 threshing wheat was recorded at Aceramic Neolithic Knossos, Crete, situated in an environment  
8 that receives similar rainfall inputs as Kouphovouno in the Sparta Basin. The Neolithic samples  
9 from Knossos ( $n=3$ ) had significantly higher  $\delta^{15}\text{N}$  values (between  $+5.1\text{‰}$  to  $+5.7\text{‰}$ ) compared  
10 to Late Bronze Age emmer samples from the same site ( $n=4$ ,  $\delta^{15}\text{N}$ :  $+3.6\text{‰}$  to  $+5.5\text{‰}$ ) (Nitsch et  
11 al. 2019).

12  
13 Apart from the archaeobotanical assemblage from 7<sup>th</sup> millennium BC Knossos (Sarpaki 1995),  
14 free-threshing wheat does not occur in significant quantities in Neolithic contexts in Greece. At  
15 Kouphovouno, it mostly occurs in grain-rich deposits, suggesting that the farmers had distinct  
16 reasons to cultivate it in the first place. It is for this reason that they may have placed higher value  
17 on it, and cultivated it under higher management intensity. Similar high-intensity management of  
18 free-threshing wheat was recorded at Aceramic Neolithic Knossos

19  
20 Three new free-threshing wheat samples (one of which, KFO50, only yielded a  $\delta^{15}\text{N}$  measurement)  
21 fall below the  $+4.9\text{‰}$  line that separates the intensively grown free-threshing wheat and less  
22 intensively grown barley. This suggest that even though the main strategy was to cultivate the two  
23 crops separately under varying degrees of intensity, occasionally, the farmers sowed free-threshing  
24 wheat in the same soils as the barley, perhaps as a risk-buffering strategy.

25  
26 Einkorn is a glume wheat that played an important role in the Neolithic farming systems of  
27 northern and southern Greece (Sarpaki 1995; Valamoti and Kotsakis 2007). At Kouphovouno, it  
28 was found in contexts generally dating to the Late Neolithic. The  $\delta^{15}\text{N}$  values of einkorn obtained  
29 in this study are systematically lower than all the  $\delta^{15}\text{N}$  values of free-threshing wheat. They overlap  
30 with some of the barley samples, but most of them are systematically lower still. With values  
31 reaching below  $0\text{‰}$ , the results indicate that this crop was grown in even less  $^{15}\text{N}$ -enriched soils

1 than barley, likely in soils that were not managed at all. It may have been intended as a fodder crop  
2 or to provide reserves (for both animals and humans) in case of shortages of the other crops.

3  
4 Nowadays, wheat is considered more demanding in terms of its soil growing requirements than  
5 barley. It can withstand drier conditions, poorer soils and a degree of salinity (Zohary et al. 2012).  
6 Einkorn (also a wheat), is now often cultivated in soils with low nutrient quality, such as in modern  
7 farms in Provence, France (Bogaard et al. 2016). Considering these nutritional requirements, the  
8 fact that free-threshing wheat was consistently cultivated in more  $^{15}\text{N}$ -enriched soils compared to  
9 both hulled barley and einkorn suggests that the farmers were aware of similar ecological  
10 adaptations and catered to the crops' distinct growing requirements. Plant stable isotopic analyses  
11 have shown, however, that this is not always the case. At the Late Bronze Age site of Archontiko  
12 in northern Greece, higher  $\delta^{15}\text{N}$  values of hulled barley suggest that this crop was grown under  
13 higher intensity compared to free-threshing wheat (Nitsch et al. 2017). This indicates that early  
14 production systems were not always optimized to the crops' nutritional requirements, and other  
15 reasons, possibly stemming from culinary or economic preferences, played a role in determining  
16 the crop management of the different cereals.

17  
18 The sample sizes of the crops investigated in this study are still too small to allow for robust  
19 investigation of chronological shifts in crop management between the Middle and the Late  
20 Neolithic at Kouphovouno. Free-threshing wheat is only represented by three samples in the Late  
21 Neolithic and einkorn is only represented by one sample in the Middle Neolithic; and the latter  
22 sample may be an outlier. Even though barley presents more samples for comparison and even  
23 though the mean  $\delta^{15}\text{N}$  values of barley from the two phases are different, there is no statistical  
24 significance to this difference. It is noteworthy that the samples which widen the range of  $\delta^{13}\text{C}$   
25 values of barley as well as all the barley and einkorn samples whose  $\delta^{15}\text{N}$  values lie below 0‰  
26 date to the Late Neolithic. This may mean that in the latter period of occupation, farmers had to  
27 seek new plots of land that were more distant and/or less naturally enriched in  $^{15}\text{N}$ . These soil  
28 conditions may have accentuated the differences in soil wetness on any given year. However, due  
29 to the limited numbers of samples in the two chronological groups, caution must be exercised when  
30 interpreting these differences.

## 6.2. Sheep and goat management

The mortality profiles of sheep and goats from Neolithic Kouphovouno suggest that their exploitation strategy either 1) changed from focus on secondary products in the Middle Neolithic to focus on meat procurement in the Late Neolithic, or 2) the farmers practiced a mixed strategy during both periods of occupation (Cantuel et al. 2008). Previous bulk bone collagen  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  measurements (on a small number of individuals) indicated subtle chronological differences in multi-annual dietary averages of these two animals (Vaiglova et al. 2014a). The data showed significant differences in  $\delta^{13}\text{C}$  values between sheep and goats during the Middle Neolithic and significant differences between their  $\delta^{15}\text{N}$  values in the Late Neolithic. Although the Middle Neolithic trend is statistically significant (keeping in mind the small sample sizes), it is no longer considered worthy of discussion in this paper, because the mean  $\delta^{13}\text{C}$  values of the two animals differ by only 0.3‰. The Late Neolithic pattern of varying  $\delta^{15}\text{N}$  values, however, is still considered meaningful, as the difference between the average  $\delta^{15}\text{N}$  values is 1.1‰. What the bone collagen data thus show is that during the Middle Neolithic, the two ovicaprid species consumed isotopically similar diets, while in the Late Neolithic, they subsisted on vegetation variably enriched in  $^{15}\text{N}$ .

One possible explanation for the variable  $\delta^{15}\text{N}$  values is that in the Late Neolithic, the animals were kept in smaller herds closer to the arable landscape, and their dietary differences were thus a result of their variable browsing/grazing adaptations. Another possibility is that the animals grazed in distinct parts of the landscape (Vaiglova et al. 2014a). The assessment of seasonal dietary and grazing patterns of these animals provides an opportunity to not only better understand this chronological shift. It will also allow us to better understand the role of the animals in the subsistence economy of the Neolithic farmers.

The results of sequential tooth enamel carbonate  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  analyses indicate that sheep and goats at this site were managed differently in their first year of life. Two sheep from the Middle Neolithic exhibit sinusoidal  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  value sequences, which are reflective of seasonal fluctuations in moisture and temperature (Gat 1980; Allison and Hughes 1983; Rozanski et al. 1993; Hartman and Danin 2010). This suggests that they consumed fresh vegetation throughout the whole year, likely by grazing within the lowlands of the Sparta Basin. Both Middle Neolithic

1 goats and the third Middle Neolithic sheep, on the other hand, exhibit flattened  $\delta^{13}\text{C}$  sequences.  
2 This indicates that they did not eat fresh local vegetation during all seasons of their first year of  
3 life. Modern sheep that have been fed on fodder in Mongolia exhibit similarly dampened intra-  
4 tooth  $\delta^{13}\text{C}$ -value sequences (Makarewicz 2014), and it is likely that the three Kouphovouno  
5 individuals with dampened  $\delta^{13}\text{C}$  value sequences spent part of the year eating vegetation that was  
6 collected during another season and stored for off-season foddering. Because obligate drinkers like  
7 sheep and goats obtain most of their body oxygen from ingested water, and because the  
8 consumption of dry fodder necessitates the ingestion of additional drinking water, the  $\delta^{18}\text{O}$   
9 sequences of these animals were not affected by their consumption of fodder. Their  $\delta^{18}\text{O}$  sequences  
10 fluctuate predictably given the changing temperatures between the wet and the dry seasons. For  
11 this reason, it is argued herein that all of the Middle Neolithic ovicaprids stayed within the lowland  
12 region during their first year of life.

13  
14 During the Late Neolithic, part of the picture changes. Due to limited preservation, only one sheep  
15 from the Late Neolithic was available for analysis. This individual (KFO212) has the widest range  
16 of  $\delta^{13}\text{C}$  values and the lowest range of  $\delta^{18}\text{O}$  values of all the animals analyzed in this study. The  
17 dampened  $\delta^{18}\text{O}$  sequence suggests that the animal moved to a different altitude for part of the year,  
18 where it gained access to vegetation with more  $^{13}\text{C}$ -enriched values (which was not available to  
19 the animals that stayed in the Sparta Basin). It may have spent part of the year at a location either  
20 closer to the coast or in the Taygetos mountains. The seasonal dietary patterns of the goats did not  
21 change in the Late Neolithic. Their flat  $\delta^{13}\text{C}$ -value sequences indicate that the animals continued  
22 to be foddered during the latter part of the site's occupation.

23  
24 Overall, the findings show that while goats were managed the same way during both periods of  
25 occupation (they were kept close to the site and foddered presumably during the dry season), sheep  
26 management was more variable. In the Middle Neolithic, some sheep were herded in the  
27 surrounding Sparta Basin, while one individual was foddered. In the Late Neolithic, the one sheep  
28 available for analysis moved to a different location for part of the year likely as part of a  
29 transhumant pastoral regime.



1 Balasse and Ambrose (2005) argue that because it is so costly and labor-intensive, farmers only  
2 resort to foddering if it is *necessary* or *facilitated*. The geomorphological reconstruction of the  
3 landscape around Kouphovouno shows that water was abundant in the Sparta Basin during the  
4 Neolithic period (Fouache et al. 2007). The fertile soils in this region would have thus offered  
5 plentiful vegetation for livestock grazing, making foddering not necessary. If the soils were less  
6 productive (N-poor) than the environmental model suggests, pasture areas would have been  
7 limited, and this would be consistent with the observation that crops in the Late Neolithic grew in  
8 poorer soils, given  $\delta^{15}\text{N}$  values below 0‰. However, goats – being browsers – are able to subsist  
9 on woody vegetation and survive in dry and marginal areas without grass cover. Sheep, on the  
10 other hand, are grazers and need more nutritious grasses to survive. So if fresh browse was limited,  
11 the expectation would be that goats were allowed to graze on woody vegetation in the surrounding  
12 areas. As this is not the case, the hydrologically active environmental model cannot be disputed.

13  
14 In the absence of an environmental explanation, the reason why the goats (and some sheep) were  
15 foddered may thus have been socio-economic: for the procurement of milk. Foddering may have  
16 been facilitated by intentional cultivation of barley and/or einkorn as fodder crops. The animals  
17 that were kept close to the site may have consumed these crops directly from the fields during the  
18 growing seasons, or as harvested fodder during the off-season.

19  
20 The high-resolution stable isotopic results suggest that the differential management of sheep and  
21 goats was likely not dictated by possible shortages in fresh graze in the local landscape. Instead, it  
22 was suited to the procurement of milk from goats (which were kept close to the site all year round)  
23 and procurement of meat from sheep (which were taken to local and more distant pasture for  
24 fattening). The indication that sheep grazed in different parts of the landscape (including more  
25 distant parts either closer to the coast or at higher altitudes), can explain the small differences in  
26 bulk collagen  $\delta^{15}\text{N}$  values of Late Neolithic ovicaprids. The results of the isotopic analyses support  
27 the zooarchaeological interpretation that farmers at Kouphovouno practiced a mixed primary and  
28 secondary products exploitation strategy throughout the Neolithic occupation of the site.

## 29 30 7. Conclusions

1 The findings from this study show that farmers at Neolithic Kouphovouno developed agropastoral  
2 management strategies that were not uniform or haphazard. Instead, they were the result of  
3 intentional decisions attuned to culinary preferences, environmental opportunities and attempts to  
4 reduce risk of crop failure.

5  
6 The three main cereal crops cultivated at Kouphovouno – free-threshing wheat, hulled barley and  
7 einkorn – were grown under distinct cultivation treatments, and the choice of cultivation treatments  
8 was attuned to the crops’ ecological adaptations. Free-threshing wheat has higher soil nutrient and  
9 water requirements than barley and einkorn. The Neolithic farmers thus managed this crop at a  
10 higher intensity (in terms of labor input per square area) than barley and einkorn. Intensive  
11 cultivation was likely carried out within 1 km of the settlement and increased the  $^{15}\text{N}$  enrichment  
12 of the soils. Einkorn was grown in the most  $^{15}\text{N}$ -depleted soils, suggesting that the farmers did not  
13 make any effort to increase the crop yields of this cereal, and may have cultivated it as a risk buffer.  
14 This species-specific crop management system was thus built around notions of higher value  
15 ascribed to free-threshing wheat (grown exclusively for human consumption) and lower value  
16 assigned to the other cereals (possibly grown as a fodder crop).

17  
18 Results of sequential tooth enamel carbonate  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values provide direct evidence that  
19 foddering was indeed an integral component of the pastoral management strategy. While goats  
20 were foddered during both phases of occupation, the management of sheep was more variable.  
21 One sheep from the Middle Neolithic was foddered, two individuals from the MN grazed on fresh  
22 vegetation in the surrounding valley and one individual from the Late Neolithic partook in trans-  
23 altitudinal mobility in search of fresh vegetation during the dry season. The sample sizes of these  
24 groups are extremely limited, so caution must be exercised with interpreting any chronological  
25 change in sheep management. Nevertheless, the conclusion can be drawn that the management of  
26 sheep and goats was distinct. The difference in early life seasonal management explains the subtle  
27 differences in the bulk bone collagen  $\delta^{15}\text{N}$  values of ovicaprids (Vaiglova et al. 2014a) and  
28 provides support for the explanation that the differences in the multi-annual diets during the Late  
29 Neolithic were the result of distinct management strategies rather than varying dietary adaptations.

In addition to providing nuanced information about the dietary behavior of these two animals, the analysis presented herein furthers our understanding of the roles of the two animals in the Neolithic subsistence strategy. Analysis of animal mortality profiles led to inconclusive results, suggesting that sheep and goat exploitation strategies may have changed between the MN and the LN, or that they were mixed in both phases of occupation. The findings from this study – which show that goats were confined to the settlement likely for supplying milk, while sheep were herded on fresh vegetation for fattening prior to slaughter for meat – support the latter scenario. Overall, the integrated stable isotopic analyses of the archaeological plant and animal remains from Neolithic Kouphovouno indicate not only that agropastoral management was flexible and diverse, but that the diversity in management was driven by cultural choices favoring consumption of free-threshing wheat and goat milk.

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### **Conflict of interest**

The authors declare that they have no conflict of interest.

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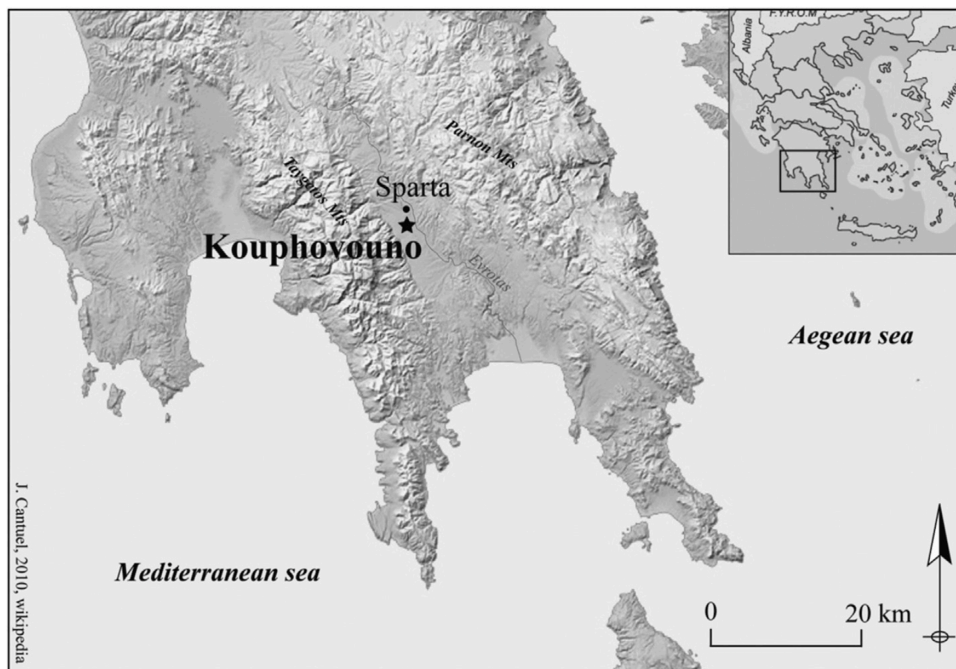
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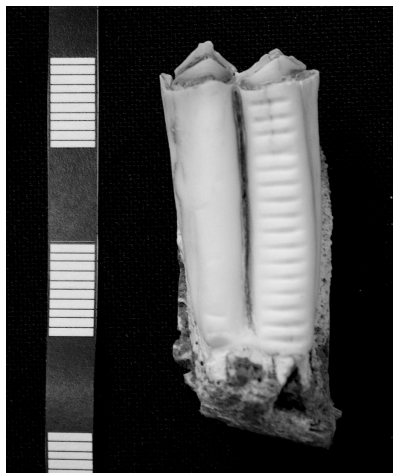
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## Figures

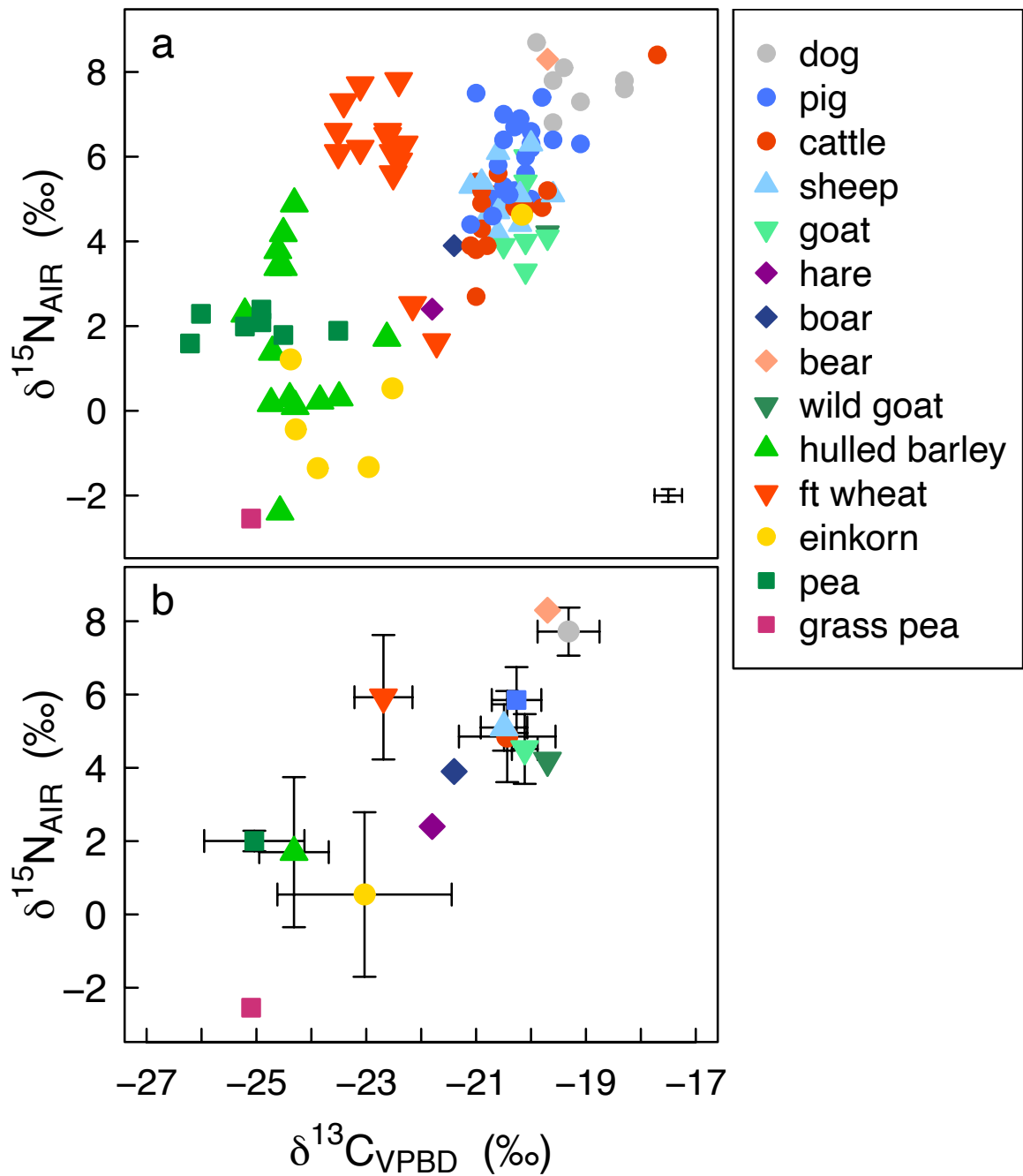
**Fig.1** Map of the Peloponnesian peninsula, southern Greece, showing the location of the Neolithic site of Kouphovouno (from Rivals et al. 2011).



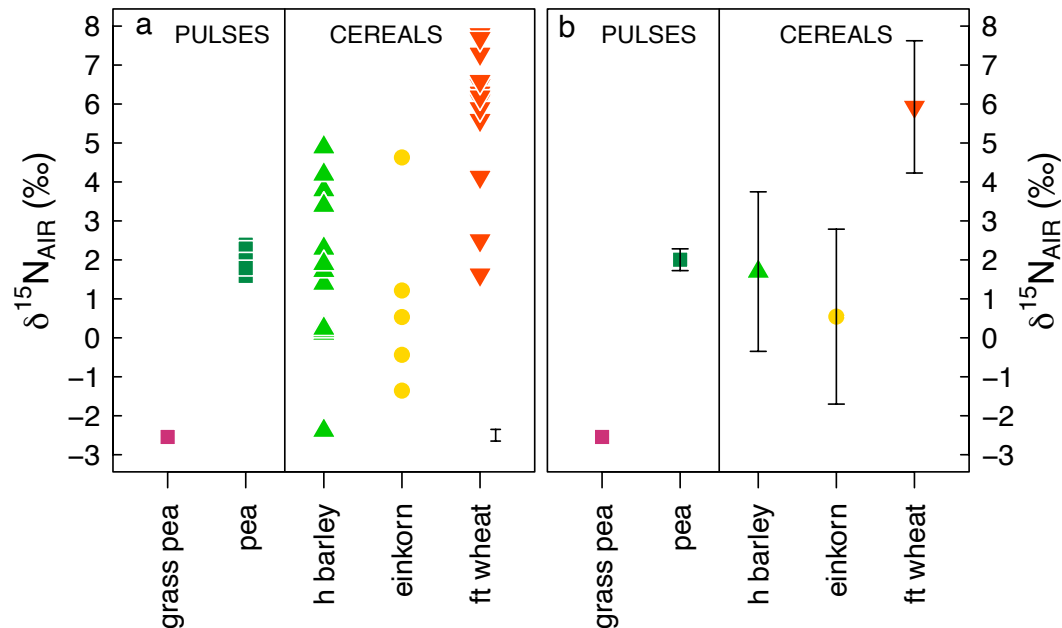
**Fig.2** Tooth enamel sampling of ovicaprid second molars carried out in stage 2 of this study.



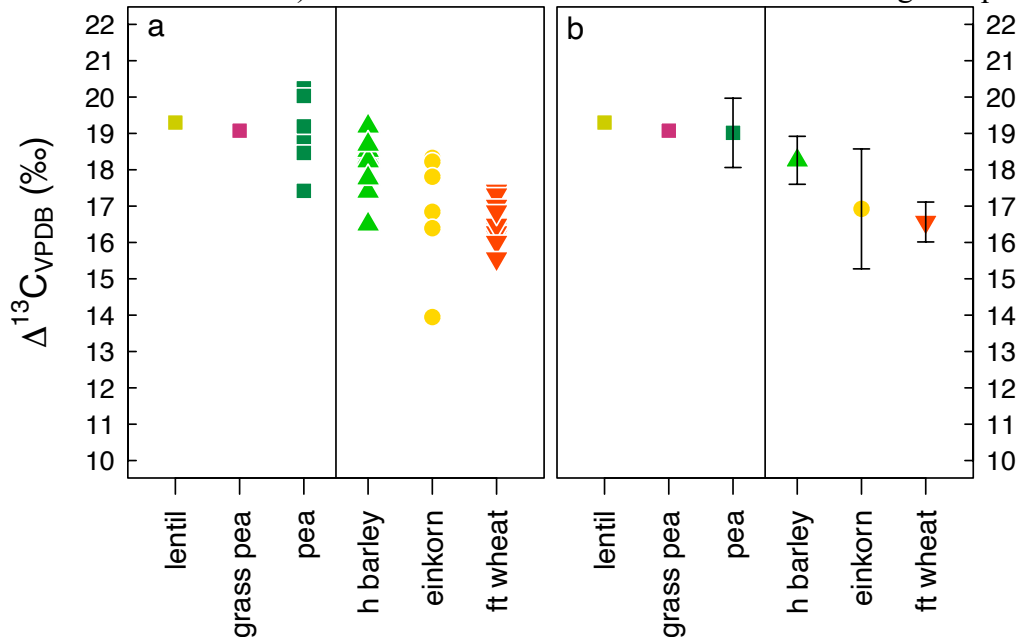
**Fig.3** All plant and animal  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values from Neolithic Kouphovouno. Samples KFO34 and KFO50 are not included, because they only yielded a reliable  $\delta^{15}\text{N}$  value. (a) individual measurements, (b) means and standard deviations of each species. The dataset includes samples measured previously (28 plants and 68 animals, Vaiglova et al. 2014a); the previous plant measurements were corrected here using the more recent charring correction (see Materials and Methods). Measurement error is shown in the bottom-right of panel a.



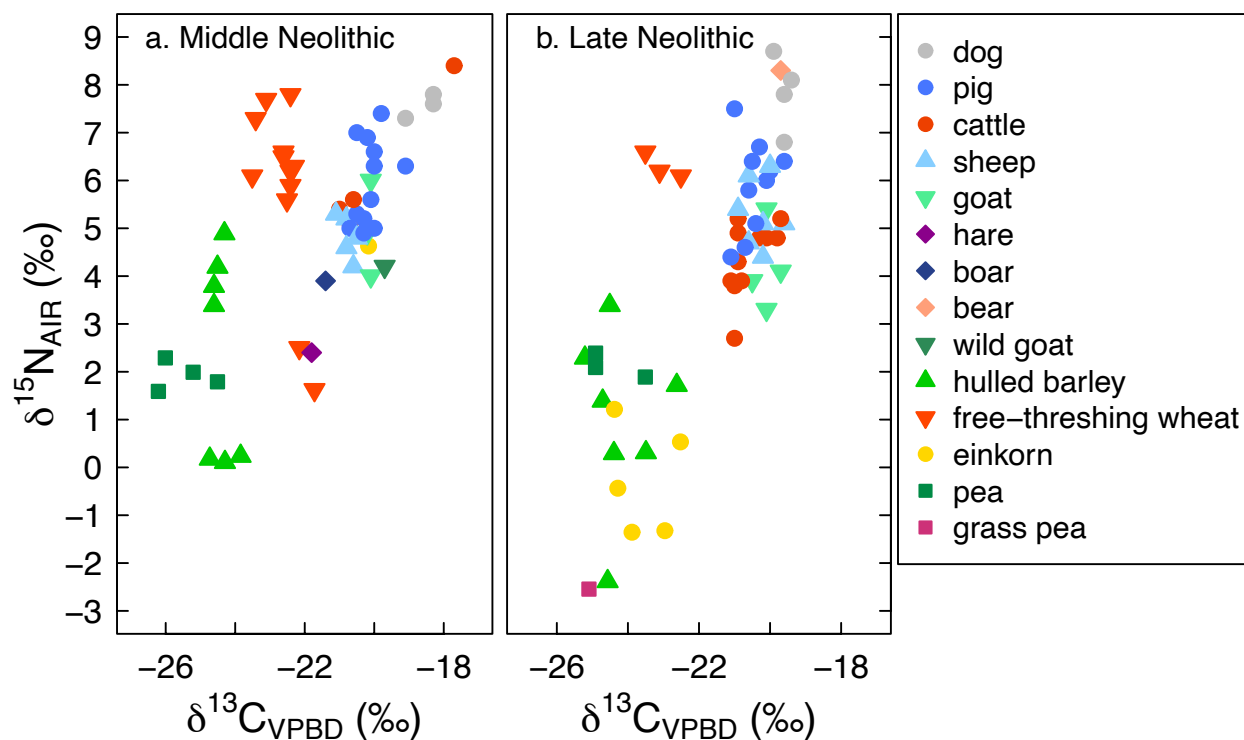
**Fig.4** All plant  $\delta^{15}\text{N}$  values from Kouphovouno. (a) all individual measurements, including KFO34 and KFO50, which are excluded from Fig.3 because they did not produce reliable  $\delta^{13}\text{C}$  measurements. (b) means and standard deviations of each species. The dataset includes samples measured previously ( $n = 28$ , Vaiglova et al. 2014a) and corrected using the more recent charring correction (see Materials and Methods). Measurement error is shown in the bottom-right of panel a.



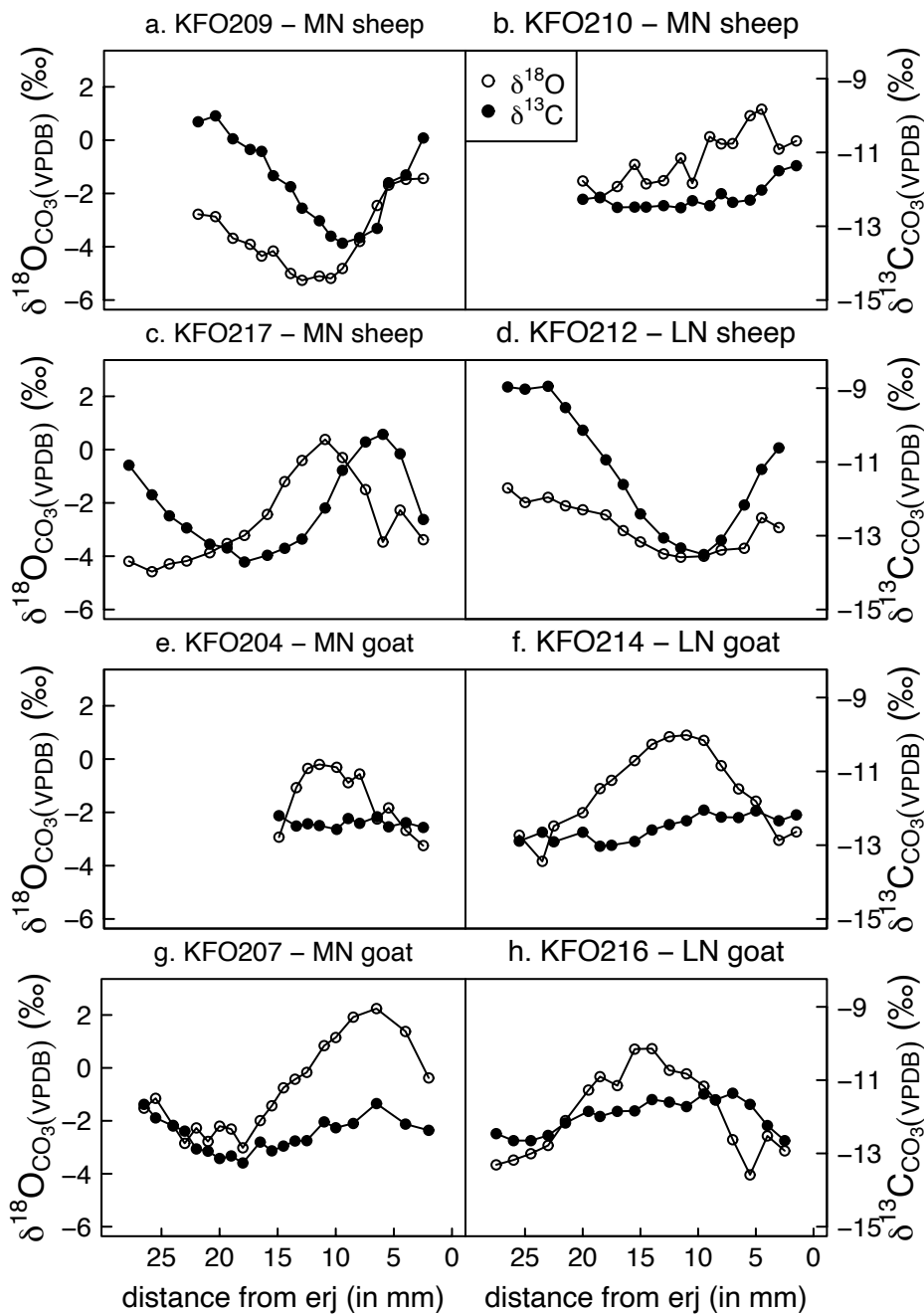
**Fig.5** All plant  $\delta^{13}\text{C}$  values from Kouphovouno. (a) all individual measurements, including KFO52, which is excluded from Fig.3 because it did not produce a reliable  $\delta^{15}\text{N}$  measurement. (b) means and standard deviations of each species. The dataset includes samples measured previously ( $n = 28$ , Vaiglova et al. 2014a) and corrected using the more recent charring correction (see Materials and Methods). Measurement error is shown in the bottom-right of panel a.



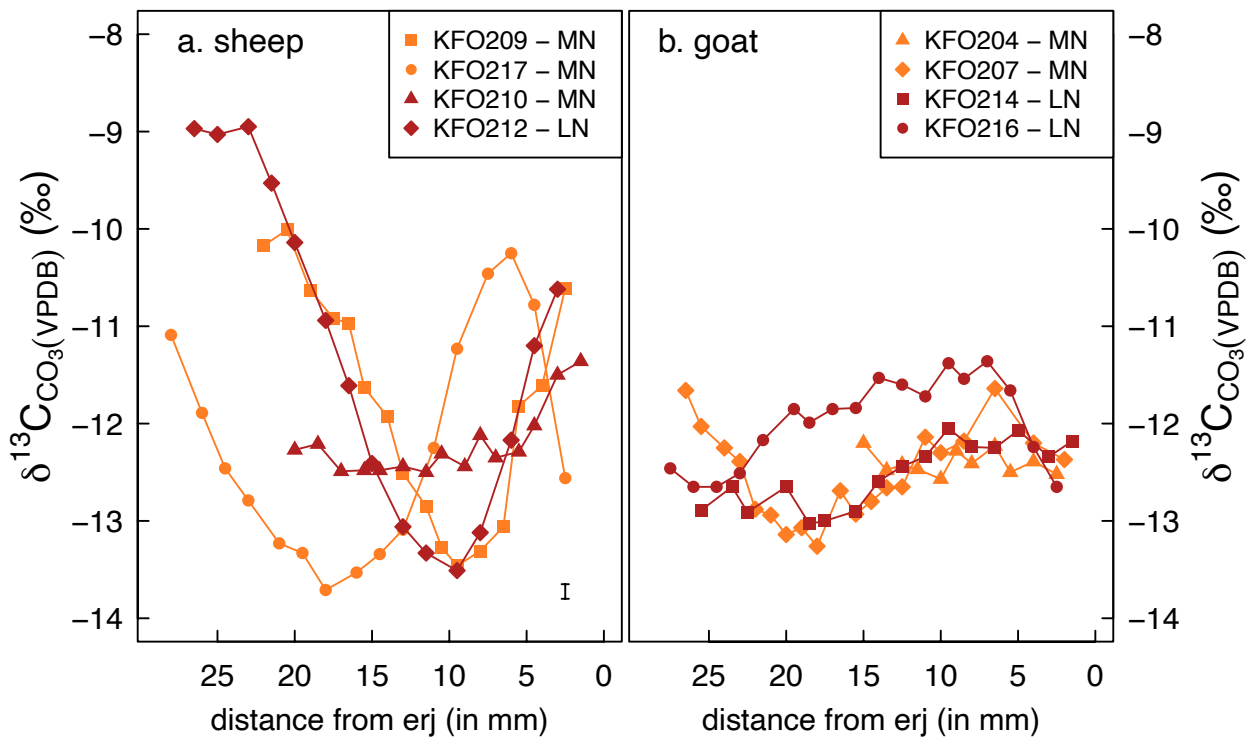
**Fig.6** All plant  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values from Kouphovouno divided according to phase of occupation.



**Fig.7** Sequential tooth enamel carbonate  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values of sheep (a–d) and goats (e–h) from Middle (MN) and Late Neolithic (LN) levels at Kouphovouno. Positions of sub-samples are recorded as distances (in mm) from the enamel root junction (erj).



**Fig.8** Sequential tooth enamel carbonate  $\delta^{13}\text{C}$  values of sheep (a) and goats (b) from Middle (MN) and Late Neolithic (LN) levels at Kouphovouno. Measurement error is shown in the bottom-right of panel a.



**Fig.9** Sequential tooth enamel carbonate  $\delta^{18}\text{O}$  measurements of sheep (a) and goats (b) from Middle (MN) and Late Neolithic (LN) levels at Kouphovouno. Measurement error is shown in the bottom-right of panel a.

