

## **Exploring Diversity in Neolithic Agropastoral Management in Mainland Greece Using Stable Isotope Analysis**

### Author

Vaiglova, Petra, Coleman, John, Diffey, Charlotte, Tzevelekidi, Vasiliki, Fillios, Melanie, Pappa, Maria, Halstead, Paul, Valamoti, Sultana Maria, Cavanagh, William, Renard, Josette, Buckley, Michael, Bogaard, Amy

### Published

2021

### Journal Title

Environmental Archaeology

### Version

Accepted Manuscript (AM)

### DOI

[10.1080/14614103.2020.1867292](https://doi.org/10.1080/14614103.2020.1867292)

### Rights statement

This is an Author's Accepted Manuscript of an article published in Environmental Archaeology, 17 Jan 2021, copyright Taylor & Francis, available online at: <https://doi.org/10.1080/14614103.2020.1867292>

### Downloaded from

<http://hdl.handle.net/10072/414029>

### Griffith Research Online

<https://research-repository.griffith.edu.au>

# Exploring diversity in Neolithic agropastoral management in mainland Greece using stable isotope analysis

Petra Vaiglova<sup>1,2\*</sup>, John Coleman<sup>3</sup>, Charlotte Diffey<sup>1</sup>, Vasiliki Tzevelekidi<sup>4</sup>, Melanie Filios<sup>5</sup>, Maria Pappa<sup>6</sup>, Paul Halstead<sup>7</sup>, Soultana Maria Valamoti<sup>8</sup>, William Cavanagh<sup>9</sup>, Josette Renard<sup>10</sup>, Michael Buckley<sup>11</sup>, Amy Bogaard<sup>1</sup>

<sup>1</sup> School of Archaeology, University of Oxford, 1 South Parks Road, Oxford, OX1 3TG, United Kingdom

<sup>2</sup> Malcolm H. Wiener Laboratory for Archaeological Science, American School of Classical Studies at Athens, 54 Souidias Street, 106 76, Greece

<sup>3</sup> Department of Classics, Cornell University, 726 University Avenue, Ithaca, NY, 14853, United States of America

<sup>4</sup> Independent researcher, Kilikias 27-29, Athens, PC 111 42, Greece

<sup>5</sup> Department of Archaeology and Paleoanthropology, University of New England, Armidale, NSW 2351, Australia

<sup>6</sup> Ephorate of Antiquities of the Thessaloniki Region, 21A Marias Kallas Street, 54645 Thessaloniki, Greece

<sup>7</sup> Department of Archaeology, University of Sheffield, Minalloy House, 0-16 Regent Street, Sheffield, S1 3NJ, United Kingdom

<sup>8</sup> School of History and Archaeology, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece

<sup>9</sup> Department of Archaeology, University of Nottingham, University Park, Nottingham NG7 2RD, UK

<sup>10</sup> Université Paul-Valéry, Montpellier 3, Route de Mende, F-34199 Montpellier, France

<sup>11</sup> Department of Earth and Environmental Sciences, Manchester Institute of Biotechnology, University of Manchester, M1 7DN, United Kingdom

\* corresponding author: [vaiglova.petra@gmail.com](mailto:vaiglova.petra@gmail.com)

Changes to affiliations (since the research on this study was conducted)

Petra Vaiglova, current affiliation: Department of Anthropology, Washington University in St Louis, 1 Brookings Drive, St Louis, 63130, United States of America

Charlotte Diffey, current affiliation: Department of Archaeology, University of Reading, Whiteknights Box 227, Reading, RG6 6AB, United Kingdom

Word count (abstract, text and captions): 8622

## ABSTRACT

New stable carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) isotopic values of charred plant and bone collagen remains from 6<sup>th</sup> mill. BCE Halai, central Greece, together with datasets from 6<sup>th</sup> mill. BCE Kouphovouno, southern Greece, and later 6<sup>th</sup>/early 5<sup>th</sup> mill. BCE Makriyalos, northern Greece, demonstrate how early farming communities in mainland Greece adapted mixed farming strategies to distinct local environmental and cultural settings. Intra-site similarities or differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of distinct crop species, along with the intra-species variabilities in stable isotopic values, are used to assess the cultivation choices that farmers at the three sites made to fulfill distinct economic goals. At Halai, farmers cultivated multiple crops under variable soil conditions, a strategy likely geared towards minimizing overall risk in a relatively arid coastal setting. At better-watered Kouphovouno, by contrast, farmers practiced strategic manuring to maximize the yield of free-threshing wheat, likely grown exclusively for human consumption and rotated with nitrogen-fixing pulses. At Makriyalos, the limited sample size of cereal remains suggests a lack of intensive manuring, in contrast to the two other sites. Assessment of which crops may have been consumed by livestock sheds further light on symbiotic relationships between crop cultivation and animal husbandry.

## KEYWORDS:

Neolithic agriculture; Aegean archaeology; stable isotopic analysis; archaeobotany; archaeozoology

## INTRODUCTION

The establishment of mixed farming, a system that incorporates crop cultivation and animal husbandry, in prehistoric Greece brought with it a series of socio-economic changes that transformed the social landscape and its ecology. A large body of research has explored the technological and economic changes that took place during the Neolithic period and the consequences it wrought in the social and political spheres of Greek societies (Perlès 2001; Kotsakis 2003; Tomkins 2004; Nanoglou 2008; Halstead 2011). While particular attention has been paid to addressing the question of *why* the adoption of agriculture happened (e.g., Ammerman and Cavalli-Sforza 1984; Renfrew 2002; Bellwood 2005; Özdoğan 2011; Clare and Weninger 2014; Mathieson et al. 2015), advances in archaeozoological and archaeobotanical methods have shed light on the equally important *how* of ancient agropastoral management strategies, in Greece

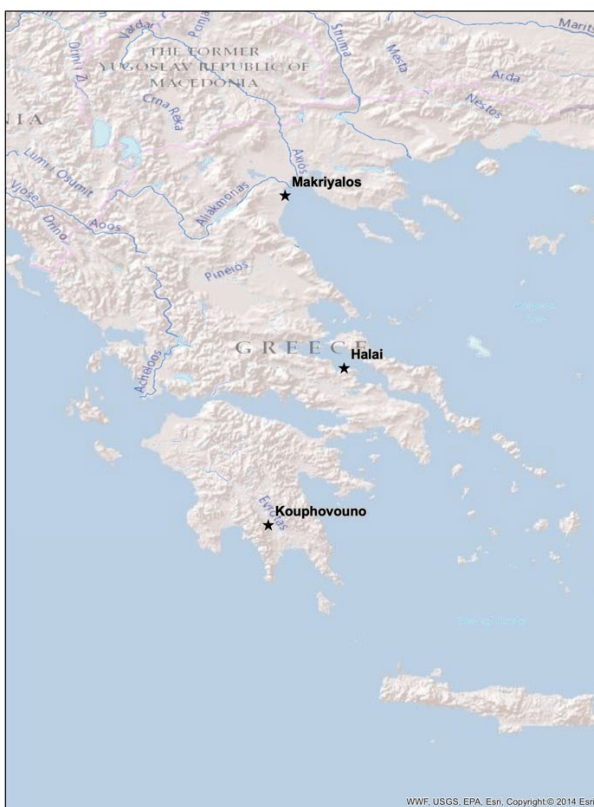
and beyond (see Halstead 1981, 1987, 2000; Valamoti 2004, 2007; Bogaard 2005; Bogaard et al. 2013).

Primary analysis of faunal and archaeobotanical assemblages from Neolithic Greece have revealed both convergence and variation: sheep are dominant, and this focus clearly circumscribed the scale of herding in what were still heavily wooded environments, whereas crop spectra are variable, with hulled wheats and barley in northern Greece contrasting with a more diverse spectrum including free-threshing wheats at some southern sites (Sarpaki 1995; Valamoti and Kotsakis 2007; Cavanagh et al. 2017; Coleman et al. 2017). Moreover, the establishment of farming in the heavily dissected Greek peninsula entailed its adaptation to variable climatic conditions (especially rainfall), settings (e.g., coastal versus inland), and soils.

Equally, the development of the Greek Neolithic, from its initial establishment in the earlier 7<sup>th</sup> millennium BCE through the 6<sup>th</sup> and the 5<sup>th</sup> millennia BCE, clearly manifests regional cultural forms and identities. The material culture at Halai exhibits close parallels to contemporary sites in the northwestern Euboean Gulf and the immediate inland regions of central Greece. Coleman et al. (2017) describe a 'Neolithic cultural interaction zone' between these areas, potentially enabling Melian obsidian worked at Halai to be distributed more widely, including to Thessaly. Ceramic styles found at Kouphovouno bear resemblance to those found across the Peloponnesian peninsula, which was occupied during the Neolithic at much lower densities than central Greece (Mee et al. 2014). At Makriyalos, painted pottery resembles that of assemblages from Thessaly, while a bowl with anthropomorphic handles reflects characteristics of Balkan material culture (Pappa et al. 2004).

The aim of this study is to explore variability in how early farmers managed agropastoral production in Neolithic mainland Greece by comparing the isotope ecology of crops and livestock in three distinct environmental and cultural settings. We use stable isotopic measurements to assess crop growing conditions and livestock diets at three sites, selected on the basis of systematic recovery of bioarchaeological remains, resulting in extensive plant and faunal assemblages: 1) Early to Late Neolithic Halai, central Greece (previously unpublished); 2) Late Neolithic Makriyalos, northern Greece (new and published data; Triantaphyllou 2001; Vaiglova et al. 2018);

and 3) Middle to Late Neolithic Kouphovouno, southern Greece (Vaiglova et al. 2014a, 2020) (Figure 1, Table 1). These are the first Greek Neolithic sites where plant, faunal, and (at Halai and Makriyalos) human stable isotopic analyses have been combined. Collectively they span much of the geographic and cultural variability of Neolithic mainland Greece and thus offer a first opportunity to assess how widely agropastoral management varied in actual practice. While this is far from a synthetic isotopic picture for the whole of Neolithic Greece, this study is a step towards developing a fine-grained, comparative perspective on the long-term evolution of farming in the centuries following its initial establishment in the region.



**Figure 1.** Location of Neolithic sites discussed in this study. Prepared using ArcGIS by Connie Lam.

## ARCHAEOLOGICAL BACKGROUND

### Halai

Halai (pronounced HAH-laye) is located on a narrow coastal strip of East Lokris on the North Euboean Gulf in central Greece and receives an average of 561 mm of rainfall per year (derived

from interpolation of average monthly climate data for 1960–1990 from the WorldClim database; Hijmans et al. 2005) (Table 1). Halai was occupied from the Early Neolithic (EN) through the Late Neolithic (LN) and later in the Archaic, Classical, Hellenistic, Roman, and Byzantine periods (Coleman et al. 1992, 1999; Facorellis and Coleman 2012). The Early–Late Neolithic occupation at Halai spanned c. 6000–5300 BCE and was divided into 5 phases: *Phase 1* (late Early Neolithic (EN)/early Middle Neolithic (MN), 6370–5810 cal BCE), *Phase 2* (earlier MN, 5870–5750 cal BCE), *Phase 3* (later MN, no <sup>14</sup>C dates but contextual estimate of 5800–5600 BCE), *Phase 4* (earlier Late Neolithic (LN) I, 5600–5480 cal BCE), and *Phase 5* (later LN I, no <sup>14</sup>C dates but contextual estimate of 5450–5300 BC) (Facorellis and Coleman 2012). The Neolithic village, stretching over an area of c. 120 m NW-SE and 30 m NE-SW, is currently located on the shore of the Bay of Atalanti, but it is inferred that during the Neolithic period, the shoreline lay some 20 m below its present position and perhaps c. 2 km distant from the site (Coleman et al. 2017). The houses were generally rectilinear structures with stone bases supporting mudbrick superstructures. In the main excavated area at the western end of the site, rectangular or sub-rectangular buildings were grouped together.

The buildings, the pottery, and the other small finds at Halai resemble those from many other sites in Central Greece, indicating likely contact (by both land and sea) between contemporary settlements across the region. For instance, Red-on-White pottery is common in the Middle Neolithic period throughout the region, as is Matt Painted pottery in the earlier Late Neolithic period. Small terracotta figurines from Halai, which were likely used in household rituals, have parallels throughout Greece. Roughly decorticated nodules of Melian obsidian were worked at Halai, and the products probably circulated to inland sites as well as seaside sites in Thessaly (Coleman et al. 2017).

Systematic sampling and flotation at Halai enabled the recovery of a large plant assemblage. Primary analysis of 131 samples, selected from well-dated contexts, shows that the most abundant crop species in the archaeobotanical assemblage was hulled barley (*Hordeum vulgare*), with glume wheats, particularly einkorn (*Triticum monococcum*), also common (Diffey and Bogaard in prep; Coleman et al. 2017). Free-threshing wheat (*Triticum aestivum*/*Triticum durum*) was less frequent, while sporadic oat (*Avena sativa*) was likely an arable weed (Zohary et al. 2012). Alongside

cereals, pulses (specifically lentil, *Lens culinaris*) and figs (*Ficus carica*) were also ubiquitous. The crop spectrum appears to have contracted over time, as evidenced by a reduction in diversity of both cereal and pulse species (Coleman et al. 2017; Diffey and Bogaard in prep).

## **Makriyalos**

Makriyalos (pronounced mak-REE-yah-los) is a Late Neolithic ‘flat extended’ settlement located in the coastal region of Pieria in Greek Macedonia and receives 443 mm rainfall per year on average (Hijmans et al. 2005) (Table 1). It was occupied in two spatially separate phases: Makriyalos I (Late Neolithic I, 5450–4950 cal BCE) and Makriyalos II (Late Neolithic II, 4950–4500 cal BCE; Maniatis and Pappa 2020) (Pappa et al. 2013; Pappa and Besios 1999; Tsokas et al. 1997). At Makriyalos I, the settlement was thinly dispersed over an area of 28 ha and was enclosed by a pair of concentric ditches. Habitation was organized into clusters of semi-subterranean pit-dwellings, which were loosely dispersed within the enclosure and served a domestic purpose. Among the number of ‘borrow pits’ that were dug within the settlement space, Pit 212 presents an exceptionally large assemblage of faunal and pottery remains, which have been interpreted to represent the remains of a series of large-scale feasting events (Pappa et al. 2004). Pottery from this phase includes black burnished and black-topped pottery, often with white painted decoration. In Makriyalos II, habitation was organized more densely over an area of 11 ha. The surviving structures consist of underground features – subterranean pits, basements, and midden areas – and place emphasis on the domestic/individual rather than the collective (Pappa 2018). Borrow pits continued to be used, but the system of enclosure ditches was abandoned. The pottery assemblage is painted (brown on cream and black on red) and bears resemblance to that found at sites in Thessaly.

The archaeobotanical assemblage was primarily composed of cereal chaff, and Valamoti (2004) argues that chaff per se or as a component of dung was used at this site as fuel in cooking installations like hearths and ovens. The only cereal types evidenced at the site as a crop are glume wheats (einkorn; emmer, *Triticum dicoccum*; and ‘new type’ glume wheat; Jones et al. 2000), with extremely rare occurrences of free-threshing wheat. Barley appears in low proportions, and pulses are represented by lentils, bitter vetch (*Vicia ervilia*), pea (*Pisum sativum*), and grass pea (*Lathyrus sativus*).

Previous bulk carbon and nitrogen stable isotopic studies have been conducted on human remains from Makriyalos I (n=18) and animal remains (wild boar, n=5; red deer, n=5; and domestic pig, n=5) from Makriyalos II (Triantaphyllou 2001) and on domestic animals (n=62) and a small number of crop grains/seeds (n=5) from Makriyalos I (Vaiglova et al. 2018). These data will be synthesized here with the new findings for a fuller spatial and temporal assessment of crop management practices.

### **Kouphovouno**

Kouphovouno (pronounced koo-FO-voo-no) is a tell site with Middle Neolithic (MN) and Late Neolithic (LN), as well as later, occupation. It is located in the Sparta Basin at the heart of the Peloponnesian peninsula in southern Greece. With an average of 718 mm/year (Hijmans et al. 2005), it receives the highest amount of rainfall of all sites considered in this paper (Table 1). Its MN-LN occupation occurs between 5800–5000 cal BCE (Cavanagh et al. 2004; Mee et al. 2014). The settlement mound spreads over an area of 4–5 ha and rises to an elevation of 5 m above the surrounding basin, reaching 200 meters above sea level (masl); it is bound to the north by the seasonal Parori stream. The Taygetos Mountains with alluvial fans on their piedmont are located 5 km to the west of the settlement, and the River Eurotas is 2.5 km to the east.

Although there are signs of continuity, important contrasts exist between the MN and LN occupation levels at Kouphovouno. The excavated MN settlement consists of small, clay-built structures (with internal area of 3.5–7 m<sup>2</sup>), separated by courtyards and passages. This configuration resembles contemporary sites in southern Greece (e.g., Lerna and probably Franchthi paralia), but contrasts with sites in central and northern Greece, such as Halai, Sesklo, and Nea Nikomedeia, which had larger buildings. Free-standing houses built and rebuilt on top of one another have been interpreted as small nuclear households, which may have had garden plots attached to them (Cavanagh et al. 2017). In addition, large samples of carbonized free-threshing wheat from Structure IV, suggest that grain was stored in this building in storage pits and jars (pithoi); which were not a common feature of the MN in southern Greece (Diffey and Board in prep). In the Late Neolithic, the settlement reduced in size, as a larger number of settlements were established across southern Greece. These changes were accompanied by a diversification of the



pottery styles: from a homogenous ceramic assemblage characterized by Urfirnis style in the MN to Black Ware, Grey Ware, Matt Painted and Polychrome styles in the Late Neolithic (Mee et al. 2014).

The archaeobotanical assemblage is primarily composed of hulled barley, followed by free-threshing wheat occurring in a number of rich contexts, and other glume wheats (einkorn and emmer) appearing in very small quantities. The relatively high incidence of free-threshing wheat at Kouphovouno presents a striking contrast to the situation in northern Greece, where farmers are argued to have rejected this crop by choice (Valamoti and Jones 2003). The results of isotopic analyses of faunal and botanical material from Kouphovouno have been published elsewhere (Vaiglova et al. 2014a, 2020) and indicate that farmers managed individual crops at varying levels of intensity – defined as investment of labor and resources per unit area (cf. Halstead 2000). Free-threshing wheat was grown more intensively in nearby plots enhanced by manure/midden refuse, and its grain was likely cultivated for human consumption only. Hulled barley and einkorn, on the other hand, were grown less intensively on more distant unmanured soils, likely to be used as mixed food/fodder crop and as a buffer against crop failure. As this study attempts to characterize diversity in Neolithic crop management strategies, the results of the previous isotopic studies from Kouphovouno are included here for comparison.

## INTERPRETATIVE FRAMEWORK

### **Plants**

Stable isotopic values of plants are primarily determined by plant physiology (which determines the manner of N<sub>2</sub> and CO<sub>2</sub> uptake) as well as soil growing conditions (which may be influenced by both natural and anthropogenic factors). Distinctions in  $\delta^{15}\text{N}$  values exist between N<sub>2</sub>-fixers like pulse crops (lentils and peas) and N<sub>2</sub>-non-fixers like cereals (barley and wheat). N<sub>2</sub>-fixers can obtain nitrogen directly from the atmosphere via symbiosis with rhizobia fungi in their roots. Their  $\delta^{15}\text{N}$  values are thus closer to 0 ‰, which is the value of AIR (Ambient Inhalable Reservoir). N<sub>2</sub>-non-fixers assimilate nitrogen from the soil, and their grain  $\delta^{15}\text{N}$  values thus reflect varying levels of soil <sup>15</sup>N enrichment (Szpak 2014; see also below). Experimental studies have shown that within these two categories of plants, crops do not exhibit species-specific differences in nitrogen fractionation. As a result, barley and bread wheat grown under the same conditions exhibit

indistinguishable  $\delta^{15}\text{N}$  values, and natural variability within a field does not generally exceed c. 2 ‰ (Bogaard et al. 2007; Fraser et al. 2011; Styring et al. 2016b).

Soil  $^{15}\text{N}$  enrichment is caused by a host of both natural factors (nutrient status, salinity, topography, water saturation; Szpak 2014; Hartman and Danin 2010) and as anthropogenic factors (application of organic matter in the form of farmyard manure or midden material; Bogaard et al. 2007; Fraser et al. 2011). In experimental and non-mechanized farming settings,  $\text{N}_2$ -non-fixers grown under varying levels of manure application recorded  $\delta^{15}\text{N}$  values between +3 and +8.5 ‰ (Fraser et al. 2011). In the same settings,  $\text{N}_2$ -fixers recorded smaller increases in  $\delta^{15}\text{N}$  than  $\text{N}_2$ -non-fixers. Notable enrichment ( $> +1$  ‰) was only recorded in fields where pulses received significant amounts of manure, such as through creation of ‘dung-soils’, *koprókhoma*, on the island of Evvia, Greece (Fraser et al. 2011). For more studies on anthropogenic influence on plant  $\delta^{15}\text{N}$  values, see Bogaard et al. (2013), Choi et al. (2002), Kanstrup et al. (2012), and (Styring et al. 2016a).

A survey of modern plants in Israel recorded differences of 12 ‰ (between  $-3$  and  $+9$  ‰) in  $\delta^{15}\text{N}$  values of unmanaged plants grown across a wide altitudinal gradient ( $-400$  to  $1000$  masl) (Hartman and Danin 2010). High  $\delta^{15}\text{N}$  values were recorded in locations that favor denitrification, the process that converts nitrogen-containing compounds into gaseous  $\text{N}_2$ , which occurs in wetland environments such as desert *wadis*, or drainage channels. Plant  $\delta^{15}\text{N}$  was also found to be negatively correlated with mean annual precipitation (MAP; Craine et al. 2009; Hartman and Danin 2010) but, due to high local variabilities, these differences can only be traced across large differences in MAP. For this reason, we argue that crops grown at the three borderline semi-arid locations investigated in this study will not record noticeable differences in  $\delta^{15}\text{N}$  caused by varying amounts of rainfall.

All of the crops discussed in this paper belong to the  $\text{C}_3$  plant category, whose  $\delta^{13}\text{C}$  values range between  $-21$  and  $-34$  ‰, with an average of  $-26.7 \pm 2.3$  ‰ (Cerling et al. 1997). In Mediterranean climates, the primary driver of  $\text{C}_3$  plant  $\delta^{13}\text{C}$  variability is water status, with plants growing in wetter soils recording more negative  $\delta^{13}\text{C}$  values than plants growing in drier/water-stressed conditions (Wallace et al. 2013). In order to account for the carbon isotopic discrimination during

photosynthesis irrespective of the  $\delta^{13}\text{C}$  value of atmospheric  $\text{CO}_2$  at the time of plant growth, plant  $\delta^{13}\text{C}$  values are converted to  $\Delta^{13}\text{C}$  values using the equation from Farquhar et al. (1989). The conversion changes the directionality of the water status, with higher  $\Delta^{13}\text{C}$  values recording wetter soil conditions, reflective primarily of the water status during the grain-/seed-filling period of cereals and pulses. Comparisons of  $\Delta^{13}\text{C}$  values of ancient agricultural crops can thus be used to assess whether crops grown in the same system received distinct watering treatments. For select case studies, see Wallace et al. (2015), Styring et al. (2017), and Flohr et al. (2011).

Due to the difficulty of disentangling natural and anthropogenic causes of soil  $^{15}\text{N}$  enrichment, our framework is not based on interpretation of absolute values. This would necessitate the establishment of a natural soil  $\delta^{15}\text{N}$  baseline, which is impossible to establish in the local environments due to recent changes caused by mechanized farming and development. Instead, our interpretations are based on 1) comparisons of  $\delta^{15}\text{N}$  values of different crop species, 2) variability in  $\delta^{15}\text{N}$  values of individual species, and 3) comparisons of contemporary crop and faunal data. Large relative differences ( $> 2\text{‰}$ ) between mean  $\delta^{15}\text{N}$  values of different crop species (such as the 4 ‰ difference between free-threshing wheat and hulled barley at Kouphovouno; Vaiglova et al. 2014a, 2020) or equally large within-species variability is considered meaningful because it is beyond any measure of natural variability reported in modern experiments or traditional farming settings (Bogaard et al. 2007; Fraser et al. 2011; Styring et al. 2016b). The interpretations are thus aimed at assessing not whether the plant  $\delta^{15}\text{N}$  values were elevated compared to the local baseline, but whether different crop species were cultivated in different soils and how the crop  $\delta^{15}\text{N}$  values compared to those of herbivore forage.

Plant  $\Delta^{13}\text{C}$  values are interpreted with reference to the ‘watering bands’ established by Wallace et al. (2013). The thresholds take into account that barley is more water-stress tolerant than wheat (Anyia et al. 2007; Jiang et al. 2006; Voltas et al. 1999) and indicate the crop’s water status during the grain-filling period. We use Wallace’s division between ‘poorly watered’ and ‘moderately watered’ plants – herein referred to as ‘optimal watering threshold’ – to assess whether the crops were cultivated under water conditions that were limiting to plant growth or not. As all three sites host a Mediterranean climate with rainfall just above (Halai, Kouphovouno) or just below (Makriyalos) the upper limit for semi-arid conditions (500 mm/yr), the crops’ water statuses can

be used to draw inferences about the likelihood that the crops were grown in rainfed soils, or whether their water status was enhanced by human choices.

## **Animals**

Bone collagen is remodeled during the lives of humans and animals primarily from the individuals' dietary intake. N only occurs in dietary protein, while C forms the backbone of all three dietary macronutrients (proteins, lipids, and carbohydrates). In individuals that are not undergoing dietary stress, bone collagen C is predominantly routed from dietary protein (Ambrose and Norr 1993; Sponheimer et al. 2003). Assimilation of dietary C and N into bone collagen is accompanied by positive isotopic fractionation, accounting for increases of c. 5 ‰ in  $\delta^{13}\text{C}$  values and 3–5‰ in  $\delta^{15}\text{N}$  values with every trophic level (DeNiro and Epstein 1981; Minagawa and Wada 1984; Hedges and Reynard 2007). Infants who are breastfed exhibit trophic level enrichment in  $\delta^{15}\text{N}$  values compared to the values of their mothers (Richards et al. 2002; Fuller et al. 2006). Comparisons of plant, animal, and human  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values from within a given ecosystem can thus be used to make assessments about which dietary sources formed significant contributions to the consumers' dietary protein intake. For a more thorough review of the method, see Lee-Thorp (2008).

## **MATERIALS AND METHODS**

This study integrates new and previously published bulk  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of charred botanical and faunal collagen samples from three sites in Neolithic Greece. New data are presented from the site of Halai (91 plant samples, 75 animal samples) and from the second phase of occupation of Makriyalos (5 plant samples, 20 animal samples). Previously published data were obtained from Kouphovouno (Vaiglova et al. 2014a, 2020) and Makriyalos (Triantaphyllou 2001, 2008; Vaiglova et al. 2018). See Table 2 for a breakdown of all samples.

The samples from Halai derive from trenches F2, F3, F4, F9, and F10, from contexts dated to all phases of occupation, herein denoted as *Late EN* (Phase 1), *Early MN* (Phase 2), *Late MN* (Phase 3), *Early MN* (Phase 4), and *Late LN* (Phase 5). See Coleman (1992) for descriptions of the excavation areas. Most of the samples come from trench F2, which provided a continuous stratigraphic sequence spanning 700 years (6000–5300 cal BCE) (Facorellis and Coleman 2012). Each sample represents a homogeneous mixture of 3 to 10 grains (or fragments thereof,

Supplementary Table S1), which enabled the maximum coverage of archaeological contexts while providing the minimum amount of low N-content (%N) material required for analysis of  $\delta^{15}\text{N}$  values. The plant samples thus represent average stable isotopic values per secure archaeological context.

The faunal samples were chosen based on which elements would provide the maximum number of individuals per species category. See Supplementary Table S1 for descriptions of all samples. Zooarchaeology by Mass Spectrometry (ZooMS), carried out at the Manchester Institute of Biotechnology on collagen samples (following Guiry and Buckley 2018), was used to confirm the identification of sheep and goat samples using published caprine peptide biomarkers (Buckley et al. 2009, 2010). Out of 22 samples that were morphologically identified to species, 16 identifications matched the ZooMS assignments. Of the 10 samples that were initially identified as sheep/goat, 5 were determined to be goats and 5 were determined to be sheep based on the ZooMS results. See Supplementary Data File S1 for example spectra of the ZooMS results.

The Makriyalos II samples come from excavation areas Th, H, and X and date to the late Late Neolithic (see Pappa and Besios 1999 for descriptions of the excavation areas). The bulk plant samples contained between 3–5 whole seeds (lentils/grass pea) or 1 nutlet (terebinth). Sampling of the faunal material took account of side of body and size of the element (*humeri* of domestic animals and *radii/tibiae* of deer) to ensure that no individual was sampled twice. Sheep and goat samples at both sites were differentiated on the basis of morphological characteristics following Boessneck et al. (1964).

Collagen was extracted from the animal bones following a modified Longin (1971) procedure described in Richards and Hedges (1999). All reported bone collagen samples have C:N ratios in the accepted range of 2.9–3.6 (DeNiro 1985). Plant samples were pre-treated with 0.5 M HCl acid for 30 min at 80°C to remove possible carbonate contamination from the soil (Vaiglova et al. 2014b).

Stable carbon and nitrogen isotopic values were measured 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 (RLAHA), University of Oxford. Plant  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were measured separately using 1–2 mg aliquots for  $\delta^{13}\text{C}$  and 1–6 mg aliquots for  $\delta^{15}\text{N}$ , depending on sample %N values. Linearity due to mass differences does not seem to have affected the plant  $\delta^{15}\text{N}$  values (see Supplementary Figure S3 for graphs of mg of N vs.  $\delta^{15}\text{N}$  and %N vs.  $\delta^{15}\text{N}$ , which, as expected due to their varying %N values, show separation between cereals and pulses).

$\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were calibrated relative to VPDB and AIR, respectively. Measurement uncertainty was assessed using the method presented in Szpak et al. (2017), which estimates standard uncertainty ( $u_c$ ) as a pooled standard deviation of precision ( $u(R_w) \pm 1 \sigma$ ) and calculates accuracy ( $u(\textit{bias}) \pm 1 \sigma$ ) using variability in calibration standards, check standards, and sample replicates. Additional details and the Microsoft Excel spreadsheets used to carry out the calculations (from Szpak et al. 2017, Appendix G) are presented in Supplementary Data Files S2 and S3 (for plant measurements) and Supplementary Data Files S4 and S5 (for bone collagen measurements).

Precision ( $u(R_w)$ ) was determined to be  $\pm 0.27 \text{‰}$  ( $\delta^{13}\text{C}$ ) and  $\pm 0.29 \text{‰}$  ( $\delta^{15}\text{N}$ ) for charred plant samples, and  $\pm 0.31 \text{‰}$  ( $\delta^{13}\text{C}$ ) and  $\pm 0.19 \text{‰}$  ( $\delta^{15}\text{N}$ ) for bone collagen. Accuracy ( $u(\textit{bias})$ ) was determined to be  $\pm 0.28 \text{‰}$  ( $\delta^{13}\text{C}$ ) and  $\pm 0.33 \text{‰}$  ( $\delta^{15}\text{N}$ ) for charred plants, and  $\pm 0.21 \text{‰}$  ( $\delta^{13}\text{C}$ ) and  $\pm 0.33 \text{‰}$  ( $\delta^{15}\text{N}$ ) for bone collagen. Using the equations from Szpak et al. (2017), the total analytical uncertainty ( $u_c$ ) was estimated to be  $\pm 0.39 \text{‰}$  ( $\delta^{13}\text{C}$ ) and  $\pm 0.44 \text{‰}$  ( $\delta^{15}\text{N}$ ) for charred plants, and  $\pm 0.37 \text{‰}$  ( $\delta^{13}\text{C}$ ) and  $\pm 0.39 \text{‰}$  ( $\delta^{15}\text{N}$ ) for bone collagen.

### **Reliability of charred plant stable isotopic data**

Charred plant samples from Halai and Makriyalos II were selected for stable isotopic analysis following criteria established through charring experiments by Charles et al. (2015) and Nitsch et al. (2015). Morphological characteristics of the grains and seeds (shape and internal morphology) were used to assess the degree of charring and only samples corresponding with the ‘optimal charring window’ around 230°C were selected for analysis. The measured plant isotopic values were corrected for the charring offset of +0.3 ‰ in  $\delta^{15}\text{N}$  values and +0.1 ‰ in  $\delta^{13}\text{C}$  values (Nitsch et al. 2015). The first set of Kouphovouno values (Vaiglova et al. 2014a) was initially corrected

for a +1.0 ‰ offset in  $\delta^{15}\text{N}$  values following Fraser et al. (2011). In the more recent publication (Vaiglova et al. 2020) and here, we follow the revised (Nitsch et al. 2015) charring correction, derived from a more comprehensive experimental study including four species of cereals and two species of pulses charred under 12 combinations of heating length and temperatures. Since our interpretations are based on large relative differences between species (differences less than 2 ‰ are not deemed meaningful), the new charring correction did not change any previous interpretations.

Szpak and Chiou (2019) suggest that the reliability of the charred plant isotopic composition can be assessed by plotting the  $\delta^{15}\text{N}$  values vs. C:N atomic ratios. This plot is shown in Supplementary Figure S2. A lack of correlation between the two suggests that the original isotopic composition was not altered through diagenesis. In addition, the data presented here do not seem to be significantly affected by ‘localized diagenesis’ (caused by, e.g., microbial degradation or other soil processes affecting some soil contexts but not others; Balzer et al. 1997; Hartman et al. 2020), since species-specific patterns emerge across multiple stratigraphic units.

Hartman et al. (2020) observed that archaeological lentil seeds from the site of Tel Dor – not selected using the morphological criteria described above – contain bacterial species capable of degrading the structure of charred plant melanoidins. The authors show that seed density (volume/mass) is negatively correlated to small increases in  $\delta^{15}\text{N}$  values, reflecting what is plausibly the well-known charring effect (Kanstrup et al. 2012; Fraser et al. 2013; Styring et al. 2013; Nitsch et al. 2015). However, the authors do not demonstrate that the bacteria can cause significant alteration to the isotopic values of the samples charred in the optimal charring window. In agreement with Szpak and Chiou (2019) and Hartman et al. (2020), we urge that future studies of plant stable isotopic values use a combination of morphological criteria and quantitative criteria to select samples appropriate for isotopic analyses.

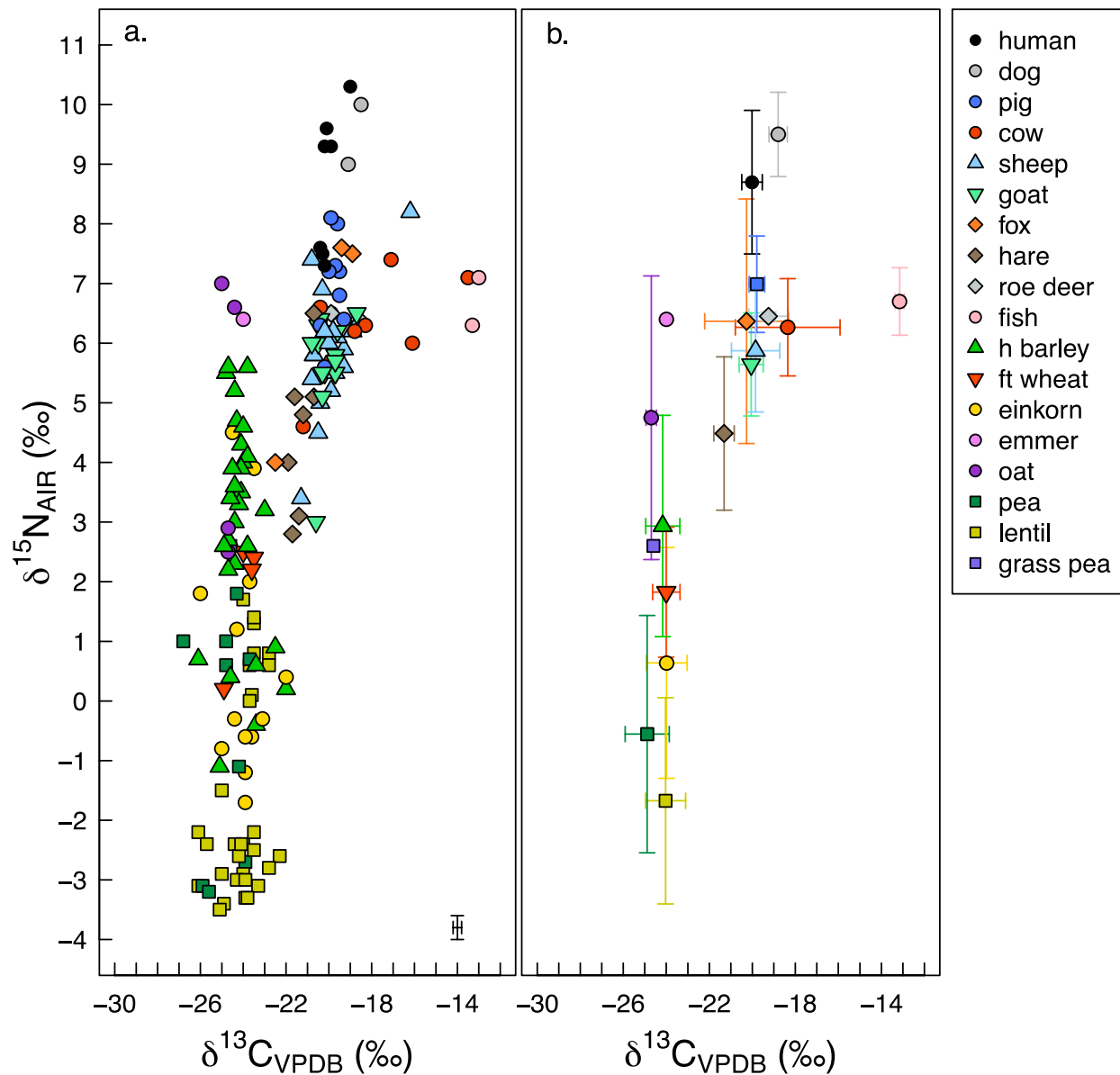
## RESULTS

### **Halai**

#### *Overall description of the plant data*

Archaeological charred plant samples from Halai exhibit large variability in  $\delta^{15}\text{N}$  values (Figure 2). Table 3 presents the summary statistics and Supplementary Table S1 details all raw data. The two cereals represented by the largest number of samples (hulled barley,  $n=29$ ; einkorn,  $n=13$ ) have  $\delta^{15}\text{N}$  values that range from  $-1.1$  to  $+5.6$  ‰ and from  $-1.7$  to  $+4.5$  ‰, respectively. The pulses record slightly lower variance, with  $\delta^{15}\text{N}$  values ranging from  $-3.2$  to  $+1.8$  ‰ (peas,  $n=9$ ) and from  $-3.5$  to  $+1.7$  ‰ (lentils,  $n=30$ ). Although the variability in plant  $\delta^{13}\text{C}$  values in Figure 2b appears to be narrow, the scale on the x-axis is necessarily compressed to include the animal samples with less negative  $\delta^{13}\text{C}$  values. The main cereals and pulses exhibit  $\delta^{13}\text{C}$  value ranges greater than 4 and 3 ‰, respectively (hulled barley:  $-26.1$  to  $-22.0$  ‰; einkorn:  $-26.0$  to  $-22.0$  ‰; pea:  $-26.8$  to  $-23.7$  ‰; lentil:  $-26.1$  to  $-22.3$  ‰).

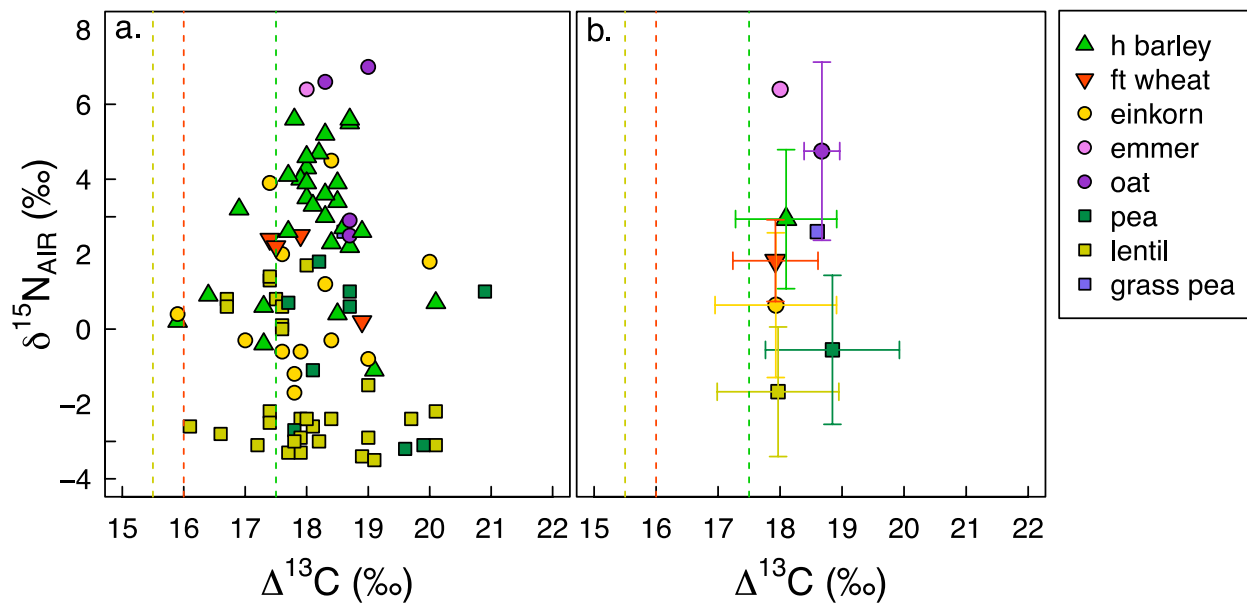




**Figure 2.**  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of all plant, animal and human samples analyzed from Halai. (a) Individual measurements, (b) species mean values and standard deviations (1 s.d.). Measurement error is shown in the bottom right-hand corner of panel ‘a’ (for calculation of measurement error, see Supplementary Data Files S2-S5); h barley = hulled barley, ft wheat = free-threshing wheat.

The three highest cereal  $\delta^{15}\text{N}$  values (+7.0, +6.6 and +6.4 ‰) belong to oat (n=2), likely an arable weed, and emmer (n=1). Free-threshing wheat, which was only available for sampling in smaller numbers (n=4), records  $\delta^{15}\text{N}$  values on the low end of the cereal spectrum, with an average  $\delta^{15}\text{N}$  value of  $+1.8 \pm 1.1$  ‰.

Figure 3 shows the measured crop  $\Delta^{13}\text{C}$  values compared to the watering thresholds established by Wallace et al. (2013). All the lentils from Halai lie above the optimal watering threshold for lentils, which suggests more-than-sufficient availability of water during the grain-filling period. All samples of free-threshing wheat and einkorn (except for one einkorn from the Late LN) lie above the optimal watering threshold for modern bread wheat. Most barley samples (24/29) lie above the threshold for barley, but five samples experienced a water status that was limiting to their growth. A multi-variate ANOVA test was used to compare the differences in  $\Delta^{13}\text{C}$  values of all the crop species measured (the data is normal: Shapiro-Wilk test,  $W = 0.974$ ,  $p = 0.069$ ; and homogeneous: Levene's test,  $F(7,83) = 1.037$ ,  $p = 0.412$ ). The results showed that there is no statistically significant difference in the  $\Delta^{13}\text{C}$  values of the crop species ( $F(7,83) = 1.296$ ,  $p = 0.263$ ).



**Figure 3.**  $\Delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of plants from Halai shown together with the ‘optimal watering threshold’ for bread wheat (red), lentils (mustard), and barley (green) (cf. Wallace et al. 2013). (a) Individual measurements, (b) species mean values and standard deviations (1 s.d.); h barley = hulled barley, ft wheat = free-threshing wheat.

#### *Overall description of the human and animal data*

The humans and animals exhibit  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values consistent with their dietary adaptations (see Figure 2). Humans ( $n=7$ ) record the highest  $\delta^{15}\text{N}$  values (from +7.3 ‰ to +10.3 ‰), followed closely by the dogs ( $n=2$ , +9.0 and +10.0‰). Two of the humans were juveniles, but these two

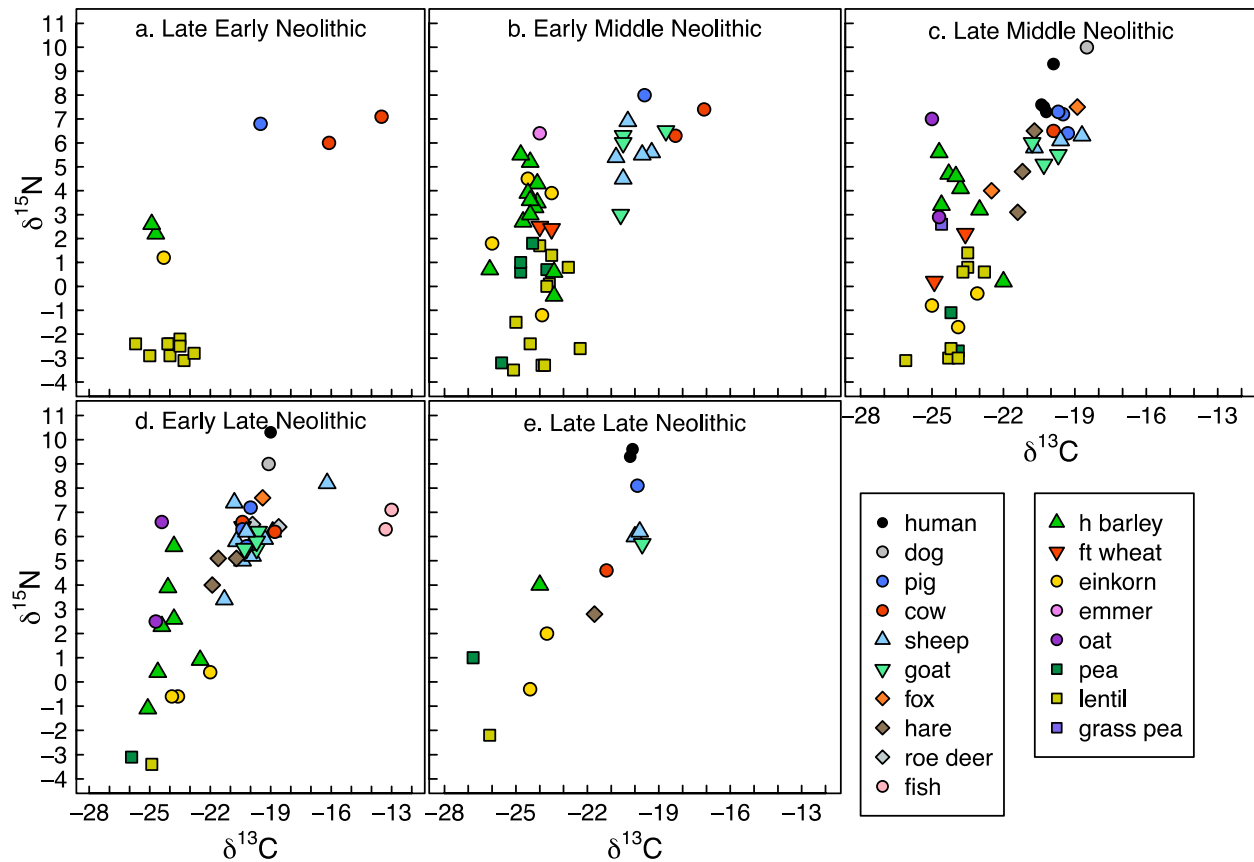
individuals do not have the highest  $\delta^{15}\text{N}$  values in the dataset (HAL256,  $\delta^{15}\text{N} = +7.6\text{‰}$ , Late MN; HAL257,  $\delta^{15}\text{N} = +9.3\text{‰}$ , Late LN). Thus, the highest human  $^{15}\text{N}$  is not a result of breastfeeding (Richards et al. 2002; Fuller et al. 2006) but rather of dietary intake.

In addition to the humans and dogs, the omnivorous pigs (n=9) and foxes (n=3) all lie a trophic level above the average values of the cultivated cereals and pulses (mean  $\delta^{15}\text{N}$  values:  $+7.0 \pm 0.8\text{‰}$ , pigs;  $+6.4 \pm 2.1\text{‰}$ , foxes; average  $\delta^{13}\text{C}$  values:  $-19.8 \pm 0.4\text{‰}$ , pigs;  $-20.3 \pm 2.0\text{‰}$ , foxes). There is no statistically significant difference between the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of the sheep (n=19) and the goats (n=14), as shown by a non-paired, unequal variance student's t-test:  $t = 0.681$ ,  $p = 0.502$  for  $\delta^{13}\text{C}$ ;  $p = 0.699$  and  $0.490$  for  $\delta^{15}\text{N}$ . With average  $\delta^{15}\text{N}$  values of  $+5.9 \pm 1.0\text{‰}$  (sheep) and  $+5.6 \pm 0.9\text{‰}$  (goats), the ovicaprids are not located a trophic level above the average values of the cultivated cereal grains or pulses. Thus, the crop parts that they are likely to have consumed in large quantities are 1) *grains* from cereals/pulses that were grown in more  $^{15}\text{N}$ -depleted soils or 2) *non-grain* components (such as chaff/stalk) from any cultivated species, as non-grain components have been shown to record lower  $\delta^{15}\text{N}$  values (by c.  $2.4\text{‰}$ ) compared to grains (Fraser et al. 2011).

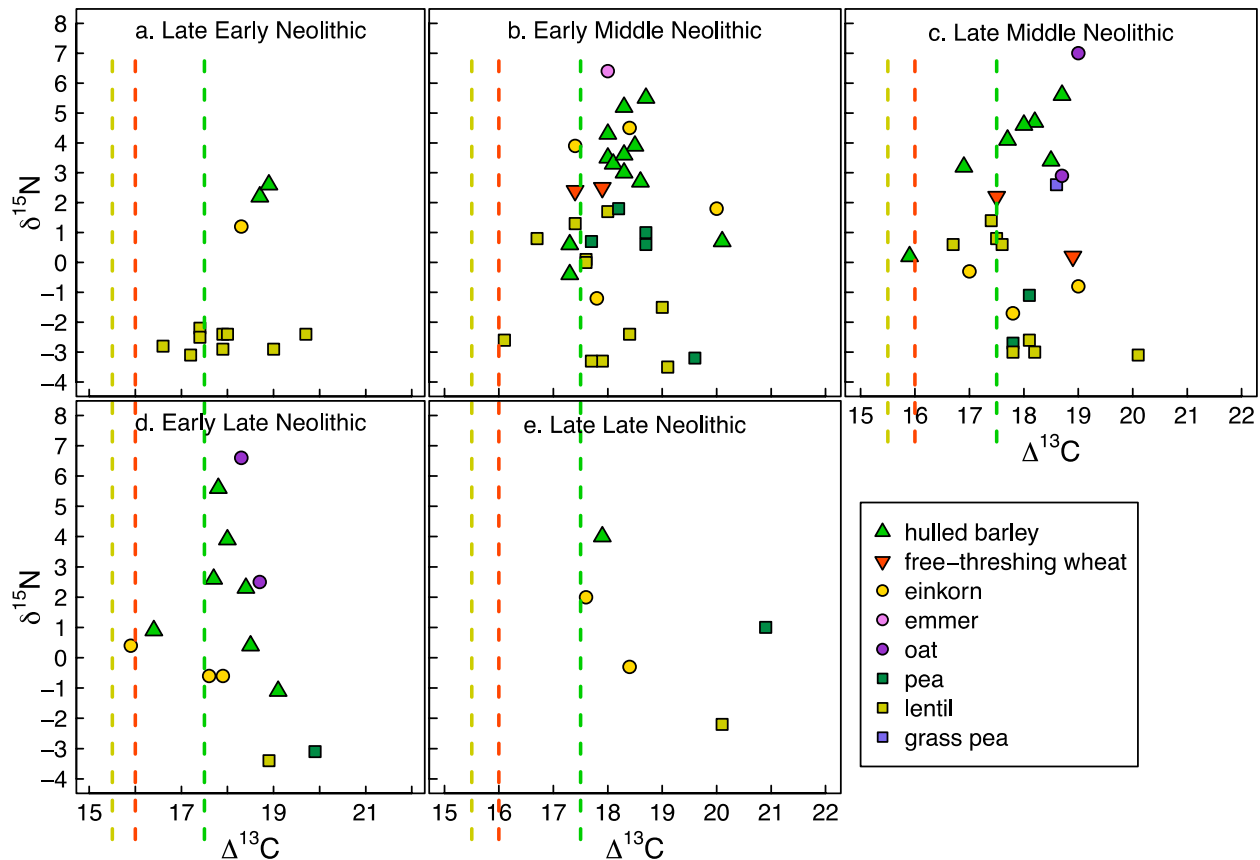
Some cattle individuals exhibit  $\delta^{13}\text{C}$  values overlapping with those of the sheep and the goats, and some reflect consumption of  $\text{C}_4$  grasses, with  $\delta^{13}\text{C}$  values above  $-18\text{‰}$ . The most  $^{13}\text{C}$ -enriched cattle samples have values almost as high as those of the two marine fish measured (fish reaching  $-13.2 \pm 0.2\text{‰}$ ).

#### *Chronological trends in the isotopic data from Halai*

Figure 4 shows all plant and animal  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values from Halai divided into the five phases of Neolithic occupation. Figure 5 shows only the plant  $\delta^{15}\text{N}$  and  $\Delta^{13}\text{C}$  data with the optimal watering thresholds. Table 4 shows the summary statistics broken down by chronology. Hulled barley, einkorn, and lentil are represented in every chronological phase and Table 5 shows the results of multivariate tests for the differences in  $\delta^{15}\text{N}$  and  $\Delta^{13}\text{C}$  values of these species through time. The results show that there are no statistically significant differences in any of the categories.



**Figure 4.** Plant and animal  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values from Halai, divided into the five phases of Neolithic occupation of the site. (a) Late Early Neolithic includes samples identified as ‘Late EN-Early MN’, (b) Early Middle Neolithic includes samples identified as ‘Earlier MN’, and ‘MN’, (c) Late Middle Neolithic includes samples identified as ‘Later MN-Earlier LN I’, ‘Later MN’, (d) Early Late Neolithic includes samples identified as ‘Earlier LN’, ‘Earlier LN I’, ‘LN I’, (e) Late Late Neolithic includes samples identified as ‘Later LN’, and ‘Later LN I’. See Supplementary Table S1 for description of contextual information of all samples; h barley = hulled barley, ft wheat = free-threshing wheat.

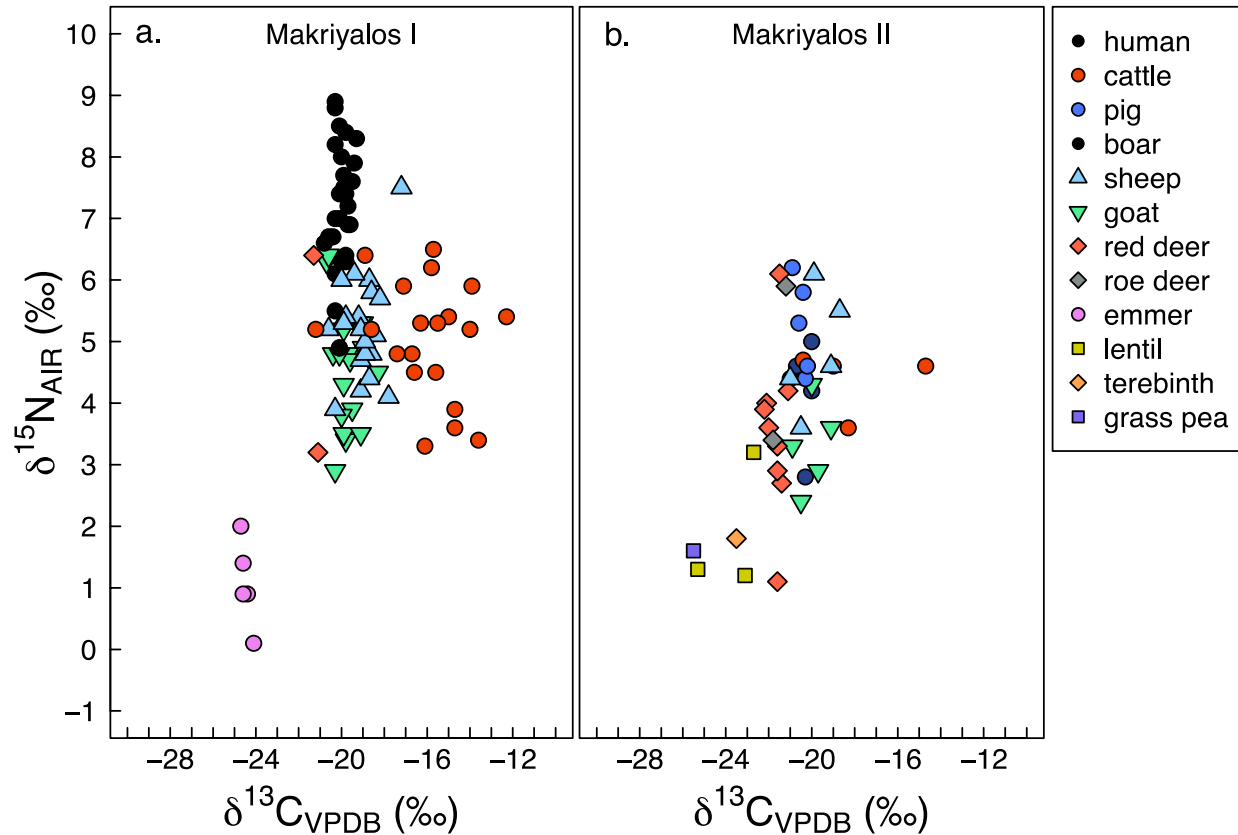


**Figure 5.** Plant  $\Delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values from Halai, divided into the five phases of Neolithic occupation of the site. ‘Optimal watering thresholds’ for bread wheat (red), lentils (mustard), and barley (green) (cf. Wallace et al. 2013) are shown in each panel. (a) Late Early Neolithic includes samples identified as ‘Late EN-Early MN’, (b) Early Middle Neolithic includes samples identified as ‘Earlier MN’, and ‘MN’, (c) Late Middle Neolithic includes samples identified as ‘Later MN-Earlier LN I’, ‘Later MN’, (d) Early Late Neolithic includes samples identified as ‘Earlier LN’, ‘Earlier LN I’, ‘LN I’, (e) Late Late Neolithic includes samples identified as ‘Later LN’, and ‘Later LN I’. See Supplementary Table S1 for description of contextual information of all samples; h barley = hulled barley, ft wheat = free-threshing wheat.

### Makriyalos

Figure 6 shows the bulk  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  data from Makriyalos I (Vaiglova et al. 2018) and Makriyalos II (this study, with data from Triantaphyllou (2008); pig,  $n=5$ ; boar,  $n=5$ ; red deer,  $n=5$ ). Supplementary Table S2 records the raw data. Due to the nature of the archaeobotanical assemblage, which is mostly composed of chaff, the plants from Makriyalos II are represented in much smaller numbers than at the other Neolithic sites. Of the three lentil samples that were measured, one has a  $\delta^{15}\text{N}$  value of +3.5 ‰ and the other two have values of +1.5 ‰ and +1.6 ‰,

similar to the one value of grass pea (+1.9 ‰). The terebinth sample provides a N<sub>2</sub>-non-fixing <sup>15</sup>N-depleted signature of +2.2 ‰.



**Figure 6.**  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of all plant, animal and human samples analyzed from Makriyalos, divided into the two phases of Neolithic occupation of the site. (a) Makriyalos I = Early Late Neolithic. (b) Makriyalos II = Late Late Neolithic. See Table 2 for references for previously published data.

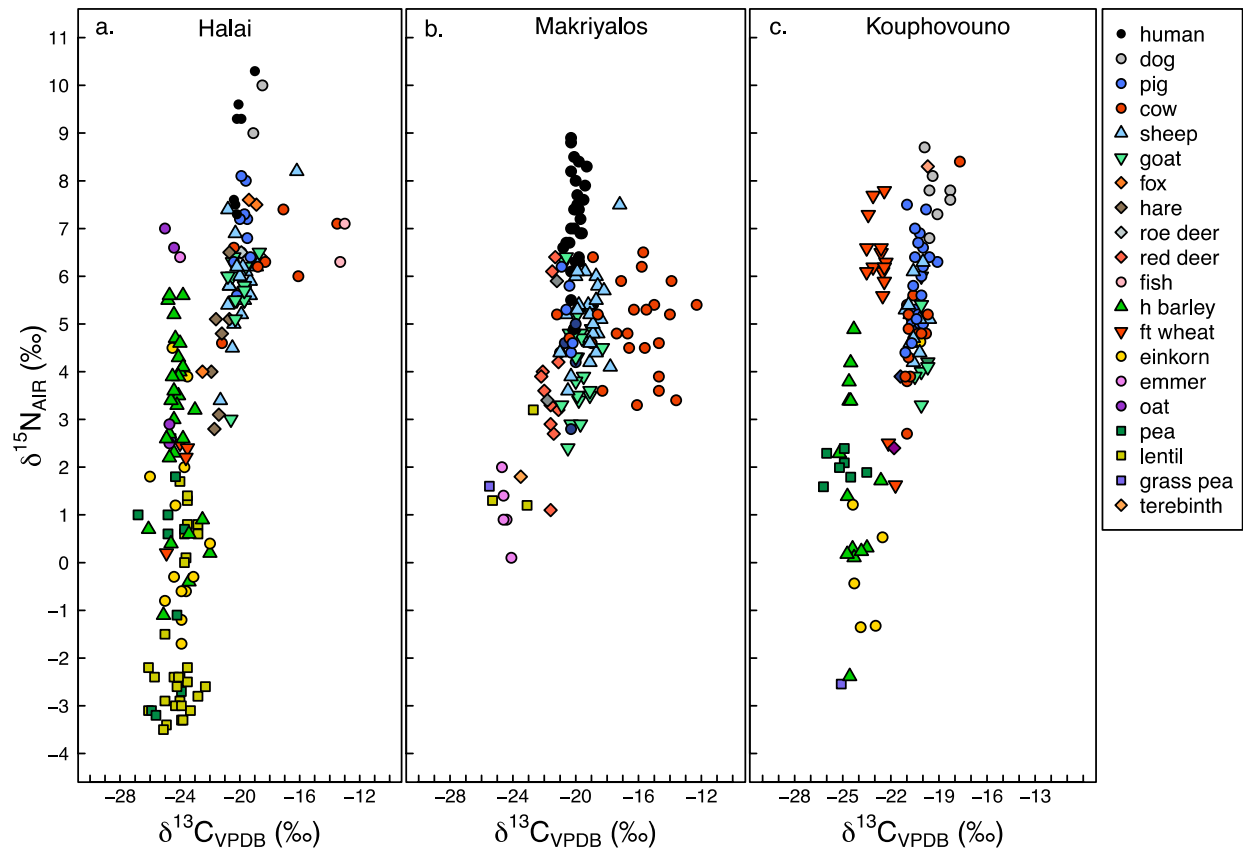
The animals exhibit narrow ranges of  $\delta^{13}\text{C}$  values and wide ranges of  $\delta^{15}\text{N}$  values. See Table 6 for summary statistics of all Makriyalos II data. The sheep and the goats have non-overlapping  $\delta^{15}\text{N}$  values, with sheep lying above and goats lying below +4.5 ‰. As such, only the sheep lie a trophic level above the measured pulses. The two roe deer measured have dissimilar  $\delta^{15}\text{N}$  values. One individual overlaps with the highest  $\delta^{15}\text{N}$  values from the site and the other individual has a value that falls in the middle of the red deer cluster. One of the cattle individuals exhibits a significant contribution of C<sub>4</sub> plants in its diet.

## DISCUSSION

### *Crop cultivation in mainland Neolithic Greece*

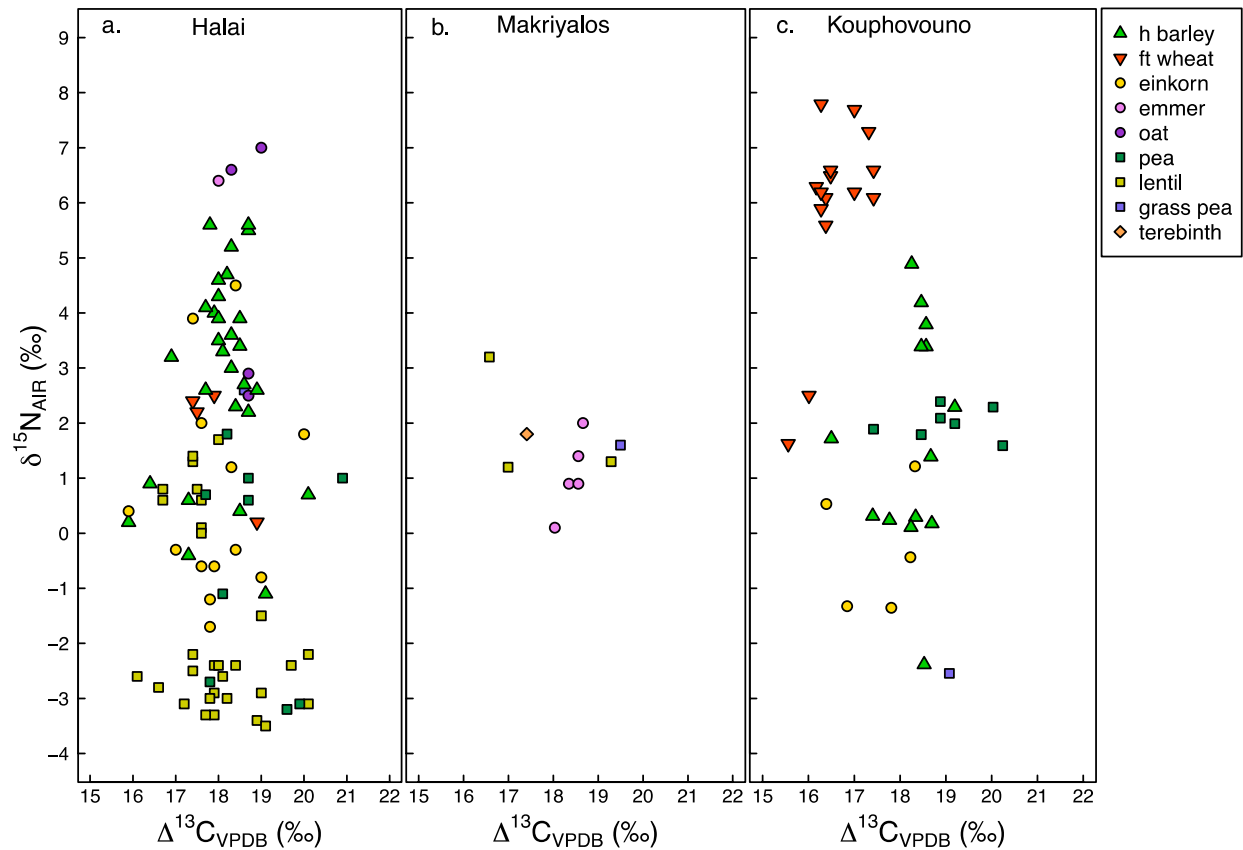
The three sites analyzed in this study afford an opportunity to assess variability in management strategies across Neolithic mainland Greece. The wide range of crop  $\delta^{15}\text{N}$  values from Halai indicates that both cereals and pulses were cultivated in variable soil conditions throughout the five phases of Neolithic occupation of the site. The variability may have been a result of a combination of natural and anthropogenic  $^{15}\text{N}$ -enrichment factors. Cultivated soils may have been located at varying distances from the coast, with more coastal plots experiencing naturally increased effects of  $^{15}\text{N}$ -enriching salinity (Heaton 1987; Ambrose 1991). In addition, some of the soils may have been enriched in  $^{15}\text{N}$  through inputs of animal manure, an intensive agricultural practice that has been shown to be in use in central and southern Europe from at least the early 6<sup>th</sup> millennium BCE on the basis of stable isotopic and weed ecological data (Bogaard 2004; Bogaard et al. 2013, 2016).

Unlike at Kouphovouno, where farmers selectively fertilized free-threshing wheat over hulled barley and einkorn (Vaiglova et al. 2014a, 2020), manure application at Halai was less species-specific (see Figure 7 and Figure 8 for comparisons of stable isotopic data from all three sites discussed in this paper). At Halai, major cereal and pulse crops (hulled barley, einkorn, lentils, and peas) were grown in soils with varying  $^{15}\text{N}$  enrichment. The less-well-represented free-threshing wheat (n=4) shows a narrower range of  $\delta^{15}\text{N}$  values (which may be due to sample size), while two emmer samples and one (weedy) oat record the highest  $\delta^{15}\text{N}$  values of all crops.



**Figure 7.** All plant and animal  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values from (a) Halai, (b) Makriyalos, (c) Kouphovouno. See Table 2 for references for previously published data; h barley = hulled barley, ft wheat = free-threshing wheat.





**Figure 8.** All plant  $\Delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values from (a) Halai, (b) Makriyalos, (c) Kouphovouno. See Table 2 for references for previously published data; h barley = hulled barley, ft wheat = free-threshing wheat.

In terms of water management, however, farmers at Halai were more strategic. As shown in Figure 3, all varieties of wheat matured in well-watered conditions, as their  $\Delta^{13}\text{C}$  values are located well above the optimal watering threshold for bread wheat. Some of the barley stands, however, grew in sub-optimal watering conditions (sub-optimal for barley, which is more drought tolerant than wheat; Zohary et al. 2012). This distinction cannot be explained by monthly amounts of rainfall and variable timing of growth of the two cereals, because barley matures earlier (Halstead and Isaakidou 2020) and would thus be expected to exhibit wetter signatures than wheat, which is harvested closer to the dry summer season. This suggests that farmers made choices that ensured that the more water-demanding wheats received more water than barley during the ripening stage of their growth cycle. They may have achieved this through strategic planting in naturally better-watered areas, planting in better-tilled soils less prone to water loss through transpiration, or

through additional watering. These choices plausibly reflect the lower mean annual rainfall at Halai compared to Kouphovouno.

Given this distinction in watering conditions, it seems unlikely that farmers at Halai practiced mixed cropping: a management strategy wherein different species of crops are grown together in the same field as a mixed crop, or ‘maslin’ (Halstead and Jones 1989; Halstead 2014:76). Subtle variations in topography of the arable landscape can cause uneven drainage (Bogaard et al. 2016), and farmers at Halai apparently sowed barley in the drier parts of the cultivated landscape. At Kouphovouno, however, all of the main cereal crops (hulled barley, free-threshing wheat, einkorn) ripened in well-watered soils (Vaiglova et al. 2014a, 2020).

Crop rotation is a strategy used by farmers to maintain the fertility of arable soils and to break crop disease cycles (Halstead 2014:201). By sowing N<sub>2</sub>-fixers (such as pulse crops) alternatively with cereals, soils can regenerate and become productive again for the nitrogen-demanding cereals. As an alternative to rotating the different crops, farmers can plant cereals and pulses together. If different plots of land are variably enriched in <sup>15</sup>N due to natural or anthropogenic causes, crops sown together would thus exhibit similar degrees of soil <sup>15</sup>N enrichment. At Kouphovouno, with mean δ<sup>15</sup>N values of +1.3 ± 0.3 ‰, peas exhibit small enrichment over their expected values of 0 ‰. This small but noticeable enrichment has been interpreted to mean that the pulses were grown in highly fertilized soils (Vaiglova et al. 2014a), as pulses in modern experimental conditions only showed positive δ<sup>15</sup>N values when grown under heavy manuring treatments (Fraser et al. 2011). The Kouphovouno peas may have been rotated with the free-threshing wheat (which grew in significantly <sup>15</sup>N-enriched soils) but not with the hulled barley or the einkorn. At Halai, hulled barley and einkorn cover a range of δ<sup>15</sup>N values from –2 to +6 ‰ and pulses cover a range from –3.5 to +2.5 ‰. It is thus possible that the cereals from the bottom half of the cereal range (between –2 and +2 ‰) were rotated with pulses from the bottom half of the pulse range (between –3.5 and –0.5 ‰) and the more <sup>15</sup>N-enriched cereals (δ<sup>15</sup>N: +2 to +6 ‰) were cultivated together with the more <sup>15</sup>N-enriched pulses (δ<sup>15</sup>N: –0.5 to +2.5 ‰). Just like the application of manure, however, this rotation schedule was not driven by species type.

Free-threshing wheat samples from Halai were measured in small numbers (n=4), but the relatively low  $\delta^{15}\text{N}$  values of these samples suggest that this crop did not enjoy the same status as it did at Kouphovouno, where it is argued to have been cultivated under especially favorable conditions, exclusively for human consumption (Vaiglova et al. 2014a). This may have been the result of a culinary choice, as people at Halai may not have shared the same preference for consuming free-threshing wheat products as did the Kouphovouno community. A complementary possibility is that the landrace of free-threshing wheat used by farmers at Halai may have been more stress-tolerant and could thus thrive in poorer soils (cf. Bogaard et al. 2016).

Just like free-threshing wheat, einkorn seems to have fulfilled distinct roles at Kouphovouno and Halai, and this is corroborated by both the archaeobotanical and the stable isotopic findings. At Kouphovouno, einkorn is found in limited quantities and records some of the lowest  $\delta^{15}\text{N}$  values indicative of  $^{15}\text{N}$ -depleted soils. This suggests that it may have been cultivated for reasons other than as a main staple crop for humans, such as fodder (both grain and chaff) and as a buffer against crop failure (Vaiglova et al. 2020). At Halai, on the other hand, where einkorn is more common (Diffey and Bogaard in prep), this crop was grown across the same range of variably  $^{15}\text{N}$ -enriched soils as the other crops. This indicates it was not assigned to a lower status, but fulfilled a more equal role to the other crops.

At Makriyalos, the only cereal whose grains were sufficiently preserved in the archaeobotanical assemblage for stable isotopic analysis was grown without intensive management. Emmer samples from the Early LN (Makriyalos I) have consistently low  $\delta^{15}\text{N}$  values (Vaiglova et al. 2018), similar to terebinth from Late LN (Makriyalos II), suggesting that both reflect an unmanured  $\delta^{15}\text{N}$  signature. While there are pulse  $\delta^{15}\text{N}$  values from Makriyalos that are consistent with soil enrichment due to manuring, the available samples are few in number and so the wider implications for the agroecology of Makriyalos are ambiguous.

#### *Human and animal diets in continental Neolithic Greece*

The plant stable isotopic results inform the interpretation of human and faunal data from Halai and Makriyalos II. Since the crops from Halai record a wide range of  $\delta^{15}\text{N}$  values, some animals fall a trophic level above some of the plants, and others do not. On the whole, omnivores including

humans, dogs, pigs, and foxes are located a trophic level above the average values of the crops. This suggests that these diets can be explained with consumption of the plant food items that have been preserved archaeologically and measured in this study. Specifically, the data provide evidence that the humans could have consumed (although not exclusively) the crops that record the highest  $\delta^{15}\text{N}$  values, around +6 ‰. As the adult human values are not located a trophic level above the animals, the data suggest – similarly to Makriyalos I (Vaiglova et al. 2018) – that the bulk of the human dietary protein did not derive from animal products but rather a mix of cereal and pulse protein.

The  $\delta^{15}\text{N}$  values of the sheep, goats, and the  $\text{C}_3$ -consuming cattle overlap with the values of the roe deer, with the mean  $\delta^{15}\text{N}$  value of their dietary input around +2 ‰ (after subtracting the 4 ‰ diet-tissue enrichment factor; Ambrose 2000; Hedges and Reynard 2007). With a mean dietary input  $\delta^{15}\text{N}$  value so low, it does not appear that most domestic herbivores at Halai were regularly grazing in or on the edges of the *manured* arable soils, even if they consumed only non-grain components of the crops. Instead, they obtained most of their graze farther away in less  $^{15}\text{N}$ -enriched parts of the landscape.

Cattle at both Halai and Makriyalos exhibit a mix of  $\text{C}_3$  and  $\text{C}_4$  dietary inputs. As argued in Vaiglova et al. (2018), the livestock at Makriyalos I may have been herded away from the settlement (cf. Valamoti 2007) in coastal salt marshes located c. 7 km from the site. This placed the antiquity of the practice of marsh-grazing of cattle – still practiced today (Halstead and Valamoti pers. comm.) – into the 6<sup>th</sup> millennium BCE. The additional cattle data from Makriyalos II measured in this study suggest that  $\text{C}_4$ -grazing of this animal continued into the Late LN, after the abandonment of the Early LN settlement. The ancient Greek name Halai (derived from the Greek word for ‘salt’) suggests that a later city built on top of the Neolithic settlement was located in close proximity to salt pans. The saline coastal environment around Halai may thus also have been hospitable to  $\text{C}_4$  vegetation, providing  $^{13}\text{C}$ -enriched graze for some of the cattle individuals. Similarly to Makriyalos, the consistently  $\text{C}_3$ -dominated human diets indicate that the  $\text{C}_4$ -fed cattle did not form a significant component of the human diets, perhaps because they were reserved as possible special (i.e., feasting) foods.

### *Diversity in agropastoral management in continental Neolithic Greece*

Overall, the data presented in this paper show that Neolithic farmers at Halai, Kouphovouno, and Makriyalos adopted both similar and distinct agro-pastoral management systems. The mixed farming economies at the three sites were small-scale and intensive (cf. Halstead 2000). The farmers strove to achieve similar goals and developed systems that allowed smaller-scale units (households or cooperating units within village ‘communes’; cf. Bogaard et al. 2017) to control production. Furthermore, they mobilized labor and resources to increase their crop yields. The ways in which they went about doing this, however, differed as their decision-making was uniquely intertwined with social constructs that dictated the demand and value of certain crops, and by opportunities and constraints determined by the surrounding environment.

At relatively well-watered Kouphovouno, manuring strategies used to enhance crop yield were driven by distinction in species. At Halai, on the other hand, all species of cereals and pulses were grown in the full range of soils available around the site, and more strategic attention was paid to ensuring adequate water availability to wheat and pulses over barley. At Makriyalos, the limited sample size suggests that emmer was grown in soils that did not receive any manure, even though the suspected grazing of sheep within the 28 ha settlement enclosure would have made manure available (Vaiglova et al. 2018). However, the possibility should not be excluded that similarly intensive treatment was accorded to other crop species that have not been preserved and are thus unavailable for analysis.

Reasons why farmers at these three sites treated their crops in distinct ways are plausibly intertwined with both social and environmental considerations. Environmental opportunities determined the types of management strategies that could be employed, but the ways in which the strategies were executed were influenced by the roles that the crops played in the farming communities. Free-threshing wheat was not cultivated widely across northern Greece (Valamoti and Jones 2003), perhaps because it was a more demanding crop than the glume wheats and barley. The fact that the farmers at Kouphovouno decided to invest in its cultivation may have been accompanied by the assignment of a special status and higher demand to it, which may have been the reason that consumption of this cereal was restricted to humans. The other two crops may have been grown for both human and animal consumption and considered lower status crops.

Preference for some crops over others may also have been determined by balancing considerations such as ease of threshing (free-threshing wheat does not need to be dehusked and is ready to use after threshing) and ease of storage (glume wheats can be stored in spikelets and are thus less prone to infestation) (Halstead 2014:136). Taste and narrative may also have played a role in shaping people's preferences of certain crops (Valamoti et al. 2017). Zohary et al. (2012:52) note that barley "is regarded as an inferior staple and a poor man's bread," likely because its cultivation signals smaller investment in resources than cereals such as wheats. In recent years, glume wheats such as einkorn and emmer have been sold at high prices because they have been marketed as ancient crops. People's preferences for or against these crops may thus be influenced by the values/biases that society imposes on the crops. Just like modern farmers and consumers, Neolithic farmers may also have placed labels and value assessments on their produce that dictated which crops were more appropriate for consumption on different social occasions.

## CONCLUSIONS

This study expands our understanding of the farming mechanisms employed by Neolithic farmers in continental Greece and suggests both environmental and cultural factors that may have contributed to the diversity of these systems. The fact that the investigation of only three case studies shows notable differences in the ways that early farmers cultivated their crops, managed their surrounding landscape, and consumed their agricultural products, suggests that early farming was strategic and sophisticated in the centuries following the introduction of farming to this region. Future research on crop cultivation strategies at other Neolithic sites in southeastern Europe is sure to shed more light on aspects of diversity and interconnection between people's subsistence systems, their environmental opportunities and cultural prescriptions.

## ACKNOWLEDGEMENTS

This work was funded by the European Research Council (PI: A Bogaard, award number 312785) and the National Environmental Research Council (PI: A Bogaard, award number NE/003761/1). We are also grateful to the support of the Royal Society for funding a fellowship to M Buckley (UF120473). Permission to carry out scientific analyses was granted by the Ephorates of Lamia (Halai), and Pieria (Makriyalos). We wish to thank the staff at the American School of Classical Studies at Athens – particularly at the Wiener Laboratory for Archaeological Science – and the

British School at Athens for their help with sampling logistics. Our thanks also go to Efi Nikita for laboratory assistance and William Cavanagh for comments on a draft of this paper.

#### DECLARATION OF INTEREST

The authors declare no conflicts of interest.

#### DISCLOSURE STATEMENT

The authors declare no financial interest that has arisen out of this research.

#### REFERENCES

- Ambrose SH (2000) Controlled diet and climate experiments on nitrogen isotope ratios of rats. In: Ambrose SH, Katzenberg MA (eds) *Biogeochemical Approaches to Paleodietary Analysis*. Kluwer Academic/Plenum, New York, pp 243–259.
- Ambrose SH (1991) Effects of diet, climate and physiology on nitrogen isotope abundances in terrestrial foodwebs. *J Archaeol Sci* 18:293–317.
- Ambrose SH, Norr L (1993) Experimental evidence for the relationship of the carbon isotope ratios of whole diet and dietary protein to those of bone collagen and carbonate. In: Lambert JB, Grupe G (eds) *Prehistoric human bone: archaeology at the molecular level*. Springer-Verlag, Berlin, pp 1–37.
- Ammerman AJ, Cavalli-Sforza LL (1984) *The Neolithic Transition and the Genetics of Populations in Europe*. Princeton University Press, Princeton.
- Anyaia AO, Slaski JJ, Nyachiro JM, et al (2007) Relationship of carbon isotope discrimination to water use efficiency and productivity of barley under field and greenhouse conditions. *J Agron Crop Sci* 193:313–323.
- Balzer A, Gleixner G, Grupe G, et al (1997) In vitro decomposition of bone collagen by soil bacteria: The implications for stable isotope analysis in archaeometry. *Archaeometry* 39:415–429.
- Bellwood P (2005) *First Farmers*. Blackwell, Oxford.
- Boessneck J, Müller H-H, Teichert M (1964) Osteologische Unterscheidungsmerkmale zwischen Schaf (*Ovis aries* Linné) und Ziege (*Capra hircus* Linné). *Kühn-Archiv* 78:1–29.
- Bogaard A (2005) ‘Garden agriculture’ and the nature of early farming in Europe and the Near East. *World Archaeol* 37:177–196.
- Bogaard A (2004) The nature of early farming in Central and South-east Europe. *Doc Praehist* 31:49–58.
- Bogaard A, Filipovic D, Charles M, et al (2017) Agricultural innovation and resilience in a long-lived early farming community: the 1500-year sequence at Neolithic-early Chalcolithic Çatalhöyük, central Anatolia. *Anatol Stud* 67:1–28.
- Bogaard A, Fraser R, Heaton T, et al (2013) Crop manuring and intensive land management by Europe’s first farmers. *Proc Natl Acad Sci* 110:12589–94.
- Bogaard A, Heaton THE, Poulton P, Merbach I (2007) The impact of manuring on nitrogen isotope ratios in cereals: archaeological implications for reconstruction of diet and crop management practices. *J Archaeol Sci* 34:335–343.
- Bogaard A, Hodgson J, Nitsch E, et al (2016) Combining functional weed ecology and crop stable isotope ratios to identify cultivation intensity: a comparison of cereal production

- regimes in Haute Provence, France and Asturias, Spain. *Veg Hist Archaeobot* 25:57–73.
- Buckley M, Collins M, Thomas-Oates J, Wilson JC (2009) Species identification by analysis of bone collagen using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry. *Rapid Commun mass Spectrom* 23:3843–3854.
- Buckley M, Whitcher Kansa S, Howard S, et al (2010) Distinguishing between archaeological sheep and goat bones using a single collagen peptide. *J Archaeol Sci* 37:13–20.
- Cavanagh W, Mee C, Renard J (2004) Sparta before Sparta: report on the Intensive Survey at Kouphovouno 1999–2000. *Annu Br Sch Athens* 99:49–128.
- Cavanagh W, Renard J, Bogaard A, et al (2017) Farming Strategies at Kouphovouno, Lakonia, in the MN-LN Periods. In: *Communities, Landscapes, and Interaction in Neolithic Greece*. pp 281–291.
- Cerling TE, Harris JM, MacFadden BJ, et al (1997) Global vegetation change through the Miocene/Pliocene boundary. *Nature* 389:153–158.
- Charles M, Forster E, Wallace M, Jones G (2015) “Nor ever lightning char thy grain”: establishing archaeologically relevant charring conditions and their effect on glume wheat grain morphology. *Sci Technol Archaeol Res* 1:1–16.
- Choi W, Lee S, Ro H, et al (2002) Natural <sup>15</sup>N abundances of maize and soil amended with urea and composted pig manure. *Plant Soil* 245:223–232.
- Clare L, Weninger B (2014) The Dispersal of Neolithic Lifeways: Absolute Chronology and Rapid Climate Change in Central and West Anatolia. In: Ozdogan M, Basgelen N, Kuniholm P (eds) *The Neolithic in Turkey. 10,500–5200 BC: Environment, Settlement, Flora, Dating, Symbols of Belief, with views from North, South, East and West*. Archaeology and Art Publications, Istanbul, pp 1–65.
- Coleman JE, Karimali E, Karali L, et al (2017) The Environment and Interactions of Neolithic Halai. In: Sarris A, Kalogiropoulou E, Kalayci T, Karimali L (eds) *Communities, Landscapes and Interaction in Neolithic Greece: Proceedings of the International Conference, Rethymno*. International Monographs in Prehistory, Ann Arbor, pp 96–124.
- Coleman JE, O’Neill K, Pomeroy M, et al (1992) Excavations at Halai, 1990-1991. *Hesperia J Am Sch Class Stud Athens* 61:265–289.
- Coleman JE, Wren PS, Quinn KM (1999) Halai: The 1992-1994 Field Seasons. *Hesperia J Am Sch Class Stud Athens* 68:285–341.
- Craine JM, Elmore AJ, Aidar MPM, et al (2009) Global patterns of foliar nitrogen isotopes and their relationships with climate, mycorrhizal fungi, foliar nutrient concentrations, and nitrogen availability. *New Phytol* 183:980–992.
- DeNiro M (1985) Postmortem preservation and alteration of in vivo bone collagen isotope ratios in relation to palaeodietary reconstruction. *Nature* 317:800–802.
- DeNiro MJ, Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals. *Geochim Cosmochim Acta* 42:495–506.
- Facorellis Y, Coleman JE (2012) Interpreting Radiocarbon Dates from Neolithic Halai, Greece. *Radiocarbon* 54:319–330.
- Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon Isotope Discrimination and Photosynthesis. *Annu Rev Plant Physiol Plant Mol Biol* 40:503–437.
- Flohr P, Müldner G, Jenkins E (2011) Carbon stable isotope analysis of cereal remains as a way to reconstruct water availability: preliminary results. *Water Hist* 3:121–144.
- Fraser R, Bogaard A, Charles M, et al (2013) Assessing natural variation and the effects of charring, burial and pre-treatment on the stable carbon and nitrogen isotope values of



- archaeobotanical cereals and pulses. *J Archaeol Sci* 40:4754–4766.
- Fraser R, Bogaard A, Heaton T, et al (2011) Manuring and stable nitrogen isotope ratios in cereals and pulses: towards a new archaeobotanical approach to the inference of land use and dietary practices. *J Archaeol Sci* 38:2790–2804.
- Fuller BT, Molleson TI, Harris D, et al (2006) Isotopic evidence for breastfeeding and possible adult dietary differences from Late/Sub-Roman Britain. *Am J Phys Anthropol* 129:45–54.
- Guiry E, Buckley M (2018) Urban rats have less variable, higher protein diets. *Proc R Soc B Biol Sci* 285:20181441.
- Halstead P (2011) Farming, material culture and ideology: repackaging the Neolithic of Greece (and Europe). In: Hadjikoimis A, Viner S (eds) *Dynamics of Neolithisation in Europe: Studies in Honour of Andrew Sherratt*. Oxbow Books, Oxford, pp 131–151.
- Halstead P (1981) Counting Sheep in Neolithic and Bronze Age Greece. In: *Pattern of the Past: Studies in Honour of David Clarke*. Cambridge University Press, Cambridge, pp 307–339.
- Halstead P (1987) Traditional and ancient rural economy in Mediterranean Europe: Plus Ça Change? *J Hell Stud* 107:77–87.
- Halstead P (2000) Land Use in Postglacial Greece: Cultural Causes and Environmental Effects. In: Halstead P, Frederick C (eds) *Landscape and Land Use in Postglacial Greece*. Sheffield Academic Press, Sheffield, pp 110–128.
- Halstead P (2014) *Two Oxen Ahead: Pre-Mechanized Farming in the Mediterranean*. John Wiley & Sons, West Sussex.
- Halstead P, Isaakidou V (2020) Pioneer farming in earlier Neolithic Greece. In: Gron KJ, Sorensen L, Rowley-Conwy P (eds) *Farmers at the Frontier: A Pan European Perspective*. Oxbow Books, Oxford, pp 77–100.
- Halstead P, Jones G (1989) Agrarian Ecology in the Greek Islands: Time Stress, Scale and Risk. *J Hell Stud* 109:41–55.
- Hartman G, Brittingham A, Gilboa A, et al (2020) Post-charring diagenetic alteration of archaeological lentils by bacterial degradation. *J Archaeol Sci* 117:105119.
- Hartman G, Danin A (2010) Isotopic values of plants in relation to water availability in the Eastern Mediterranean region. *Oecologia* 162:837–52.
- Heaton THE (1987) The  $^{15}\text{N}/^{14}\text{N}$  ratios of plants in South Africa and Namibia: relationship to climate and coastal/saline environments. *Oecologia* 74:236–246.
- Hedges REM, Reynard LM (2007) Nitrogen isotopes and the trophic level of humans in archaeology. *J Archaeol Sci* 34:1240–1251.
- Hijmans RJ, Cameron SE, Parra JL, et al (2005) Very high resolution interpolated climate surfaces for global land areas. *Int J Climatol* 25:1965–1978.
- Jiang Q, Roche D, Hole DJ (2006) Carbon isotope discrimination of two-rowed and six-rowed barley genotypes under irrigated and non-irrigated field conditions. *Can J Plant Sci* 86:433–441.
- Jones G, Valamoti S, Charles M (2000) Early crop diversity: A ‘new’ glume wheat from northern Greece. *Veg Hist Archaeobot* 9:133–146.
- Kanstrup M, Thomsen IK, Mikkelsen PH, Christensen BT (2012) Impact of charring on cereal grain characteristics: linking prehistoric manuring practice to  $\delta^{15}\text{N}$  signatures in archaeobotanical material. *J Archaeol Sci* 39:2533–2540.
- Kotsakis K (2003) From the Neolithic side: the Mesolithic/Neolithic interface in Greece. *Br Sch Athens Stud* 10:217–221.
- Lee-Thorp JA (2008) On Isotopes and Old Bones. *Archaeometry* 50:925–950.

- Longin R (1971) New Method of Collagen Extraction for Radiocarbon Dating. *Nature* 230:241–242.
- Maniatis Y, Pappa M (2020) Radiocarbon Dating of the Neolithic Settlement at Makriyalos, Pieria, North Greece. *Radiocarbon* 62:467–483.
- Mathieson I, Lazaridis I, Rohland N, et al (2015) Genome-wide patterns of selection in 230 ancient Eurasians. *Physiol Behav* 176:139–148.
- Mee C, Cavanagh B, Renard J (2014) The Middle–Late Neolithic Transition At Kouphovouno. *Annu Br Sch Athens* 1–31.
- Minagawa M, Wada E (1984) Stepwise enrichment of  $^{15}\text{N}$  along food chains: Further evidence and the relation between  $\delta^{15}\text{N}$  and animal age. *Geochim Cosmochim Acta* 48:1135–1140.
- Nanoglou S (2008) Building biographies and households: Aspects of community life in Neolithic northern Greece. *J Soc Archaeol* 8:139–160.
- Nitsch EK, Charles M, Bogaard A (2015) Calculating a statistically robust  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  offset for charred cereal and pulse seeds. *Sci Technol Archaeol Res* 1:1–8.
- Özdoğan M (2011) Archaeological Evidence on the Westward Expansion of Farming Communities from Eastern Anatolia to the Aegean and the Balkans. *Curr Anthropol* 52:S415–S430.
- Pappa M (2018) Transformations of space in the Late Neolithic settlements of northern Greece: review of the evidence from Makriyalos and Thermi. In: Dietz S, Mavridis F, Tankosic Z, Takaoglu T (eds) *Communities in transition. The Circum-Aegean Area During the 5th and 4th millennia BC*, Monographs. Oxbow, Oxford, pp 211–216.
- Pappa M, Besios M (1999) The Neolithic Settlement at Makriyalos, Northern Greece: Preliminary Report on the 1993-1995 Excavations. *J F Archaeol* 26:177–195.
- Pappa M, Halstead P, Kotsakis K, et al (2013) The Neolithic site of Makriyalos, northern Greece. In: Voutsaki S, Valamoti SM (eds) *Diet, Economy and Society in the Ancient Greek World: Towards a Better Integration of Archaeology and Science*. Peeters, Leuven, pp 77–88.
- Pappa M, Halstead P, Kotsakis K, Urem-Kotsou D (2004) Evidence for Large-scale Feasting at Late Neolithic Makriyalos, Northern Greece. In: Halstead P, Barrett JC (eds) *Food, cuisine and society in prehistoric Greece*. Oxbow Books, Oxford, pp 16–44.
- Perlès C (2001) *The Early Neolithic in Greece*. Cambridge University Press, Cambridge.
- Renfrew C (2002) The emerging synthesis: the archaeogenetics of farming/language dispersals and other spread zones. In: Bellwood P, Renfrew C (eds) *Examining the farming/language dispersal hypothesis*. McDonald Institute for Archaeological Research, Cambridge, pp 3–17.
- Richards MP, Hedges REM (1999) Stable Isotope Evidence for Similarities in the Types of Marine Foods Used by Late Mesolithic Humans at Sites Along the Atlantic Coast of Europe. *J Archaeol Sci* 26:717–722.
- Richards MP, Mays S, Fuller BT (2002) Stable carbon and nitrogen isotope values of bone and teeth reflect weaning age at the Medieval Wharram Percy site, Yorkshire, UK. *Am J Phys Anthropol* 119:205–210.
- Sarpaki A (1995) Toumba Balomenou, Chaeronia: plant remains from the Early and Middle Neolithic levels. In: Kroll R, Pasternak R (eds) *Res Archaeobotanicae, International Workgroup for Palaeoethnobotany, Proceedings of the 9th Symposium*. Oetker-Vosges, Kiel, pp 5–15.
- Sponheimer M, Robinson T, Ayliffe L, et al (2003) Nitrogen isotopes in mammalian herbivores: hair  $\delta^{15}\text{N}$  values from a controlled feeding study. *Int J Osteoarchaeol* 13:80–87.

- Styring A, Maier U, Stephan E, et al (2016a) Cultivation of choice: new insights into farming practices at Neolithic lakeshore site. *Antiquity* 90:95–110.
- Styring AK, Ater M, Hmimsa Y, et al (2016b) Disentangling the effect of farming practice from aridity on crop stable isotope values: A present-day model from Morocco and its application to early farming sites in the eastern Mediterranean. *Anthr Rev* 3:2–22.
- Styring AK, Charles M, Fantone F, et al (2017) Isotope evidence for agricultural extensification reveals how the world's first cities were fed. *Nat Plants* 3:17076.
- Styring AK, Manning H, Fraser RA, et al (2013) The effect of charring and burial on the biochemical composition of cereal grains: investigating the integrity of archaeological plant material. *J Archaeol Sci* 40:4767–4779.
- Szpak P (2014) Complexities of nitrogen isotope biogeochemistry in plant-soil systems: implications for the study of ancient agricultural and animal management practices. *Front Plant Sci* 5:1–19.
- Szpak P, Chiou KL (2019) A comparison of nitrogen isotope compositions of charred and desiccated botanical remains from northern Peru. *Veg Hist Archaeobot* 29:527–538.
- Szpak P, Metcalfe JZ, Macdonald RA (2017) Best practices for calibrating and reporting stable isotope measurements in archaeology. *J Archaeol Sci Reports* 13:609–616.
- Tomkins P (2004) Filling in the 'Neolithic background': social life and social transformation in the Aegean before the Bronze Age. In: Barrett J, Halstead P (eds) *The emergence of civilization revisited*. Oxbow Books, Oxford, pp 38–63.
- Triantaphyllou S (2001) *A Bioarchaeological Approach to Prehistoric Cemetery Populations from Central and Western Greek Macedonia*. British Archaeological Reports Limited, Oxford.
- Triantaphyllou S (2008) Living with the Dead: A Re-Consideration of Mortuary Practices in the Greek Neolithic. In: Isaakidou V, Tomkins P (eds) *Escaping the Labyrinth*. Sheffield Studies in Aegean Archaeology, Sheffield, pp 136–154.
- Tsokas GN, Sarris A, Pappa M, et al (1997) A large-scale magnetic survey in Makrygialos (Pieria), Greece. *Archaeol Prospect* 4:123–137.
- Vaiglova P, Bogaard A, Collins M, et al (2014a) An integrated stable isotope study of plants and animals from Kouphovouno, southern Greece: a new look at Neolithic farming. *J Archaeol Sci* 42:201–215.
- Vaiglova P, Gardeisen A, Buckley M, et al (2020) Further insight into Neolithic agricultural management at Kouphovouno, southern Greece : expanding the isotopic approach. *Archaeol Anthropol Sci* 12:e43.
- Vaiglova P, Halstead P, Pappa M, et al (2018) Of cattle and feasts: Multi-isotope investigation of animal husbandry and communal feasting at Neolithic Makrygialos, northern Greece. *PLOS One* e0194474.
- Vaiglova P, Snoeck C, Nitsch E, et al (2014b) Impact of contamination and pre-treatment on stable carbon and nitrogen isotopic composition of charred plant remains. *Rapid Commun Mass Spectrom* 28:2497–2510.
- Valamoti S-M, Jacomet S, Stika H-P, Heiss AG (2017) The PLANTCULT Project: identifying the plant food cultures of ancient Europe. *Antiquity* 91:1–7.
- Valamoti SM (2004) *Plants and People in Late Neolithic and Early Bronze Age Northern Greece: an Archaeobotanical Investigation*. British Archaeological Reports Limited, Oxford.
- Valamoti SM (2007) Detecting seasonal movement from animal dung: an investigation in

- Neolithic northern Greece. *Antiquity* 81:1053–1064.
- Valamoti SM, Jones G (2003) Plant Diversity and Storage at Mandalo, Macedonia, Greece: Archaeobotanical Evidence from the Final Neolithic and Early Bronze Age. *Annu Br Sch Athens* 98:1–35.
- Valamoti SM, Kotsakis K (2007) Transitions to agriculture in the Aegean: the archaeobotanical evidence. In: Colledge S, Connolly J (eds) *Origins and Spread of Domestic Plants in Southwest Asia and Europe*. Left Coast Press, Walnut Creek, pp 75–91.
- Voltas J, Romagosa I, Lafarga A, et al (1999) Genotype by environment interaction for grain yield and carbon isotope discrimination of barley in Mediterranean Spain. *Aust J Agric Res* 50:1263–1271.
- Wallace M, Jones G, Charles M, et al (2013) Stable carbon isotope analysis as a direct means of inferring crop water status and water management practices. *World Archaeol* 45:388–409.
- Wallace MP, Jones G, Charles M, et al (2015) Stable Carbon Isotope Evidence for Neolithic and Bronze Age Crop Water Management in the Eastern Mediterranean and Southwest Asia. *PLOS One* 10:e0127085.
- Zohary D, Hopf M, Weiss E (2012) *Domestication of plants in the Old World: the origin and spread of domesticated plants in south-west Asia, Europe, and the Mediterranean Basin*, 4th edn. Oxford University Press, Oxford.

## Tables

**Table 1** Environmental characteristics of sites included in this study.

Site	Altitude (masl)	Average rainfall	Temperature	Soil type
Halai	5	561 mm/yr	11-22°C	Neogene alluvium
Makriyalos	68	443 mm/yr	9-31°C	marls, paleosols
Kouphouvouno	200	718 mm/yr	15-31°C	fertile loams

**Table 2** Description of all samples presented in this study.

	Plant samples		Reference	Animal samples	
	species	n =		species	n =
Halai	hulled barley	29	this study	human	7
	free-threshing wheat	4	this study	dog	2
	einkorn	13	this study	pig	9
	emmer	1	this study	cattle	9
	oat	4	this study	sheep	19
	lentil	30	this study	goat	14
	pea	9	this study	fox	4
	grass pea	1	this study	hare	7
	<i>total</i>	<i>91</i>		roe deer	2
				fish	2
			<i>total</i>	<i>75</i>	
Makriyalos II	lentil	3	this study	cattle	4
	grass pea	1	this study	sheep	5
	terebinth	1	this study	goat	5
	<i>total</i>	<i>5</i>		pig	5
				boar	5
				red deer	2
				red deer	5
				roe deer	2
				<i>total</i>	<i>33</i>
Makriyalos I	emmer	5	Vaiglova et al. (2018)	human	12
	<i>total</i>	<i>5</i>		human	18
				cattle	20

			sheep	20
			goat	19
			red deer	3
			<i>total</i>	92

Kouphovouno	free-threshing wheat	13	Vaiglova et al. (2014a)	cattle	15
	free-threshing wheat	4	Vaiglova et al. (2020)	dog	7
	hulled barley	7	Vaiglova et al. (2014a)	sheep	12
	hulled barley	8	Vaiglova et al. (2020)	goat	7
	einkorn	6	Vaiglova et al. (2020)	pig	23
	pea	7	Vaiglova et al. (2014a)	wild boar	1
	grass pea	1	Vaiglova et al. (2020)	wild goat	1
	lentil	1	Vaiglova et al. (2014a)	hare	1
	<i>total</i>	47		bear	1
				<i>total</i>	68

**Table 3** Summary statistics for all new plant and animal  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values from Halai presented in this study. SD refers to standard deviation.

species	n =	$\delta^{15}\text{N}$ max	$\delta^{15}\text{N}$ min	$\delta^{15}\text{N}$ average	$\delta^{15}\text{N}$ SD	$\delta^{15}\text{N}$ range	$\delta^{13}\text{C}$ max	$\delta^{13}\text{C}$ min	$\delta^{13}\text{C}$ average	$\delta^{13}\text{C}$ SD	$\delta^{13}\text{C}$ range	$\Delta$ n
hulled barley	29	5.6	-1.1	2.9	1.9	6.7	-22.0	-26.1	-24.2	0.8	4.1	2
free-threshing wheat	4	2.5	0.2	1.8	1.1	2.3	-23.5	-24.9	-24.0	0.6	1.4	1
einkorn	13	4.5	-1.7	0.6	1.9	6.2	-22.0	-26.0	-24.0	1.0	4.0	2
emmer	1	6.4	6.4	6.4	-	0.0	-24.0	-24.0	-24.0	-	0.0	1
oat	4	7	2.5	4.8	2.4	4.5	-24.4	-25.0	-24.7	0.2	0.6	1
lentil	30	1.7	-3.5	-1.7	1.7	5.2	-22.3	-26.1	-24.0	0.9	3.8	2
pea	9	1.8	-3.2	-0.6	2.0	5.0	-23.7	-26.8	-24.9	1.0	3.1	2
grass pea	1	2.6	2.6	2.6	-	0.0	-24.6	-24.6	-24.6	-	0.0	1
sheep	19	8.2	3.4	5.9	1.0	4.8	-16.2	-21.3	-19.8	1.1	5.1	
goat	14	6.5	3	5.6	0.9	3.5	-18.7	-20.8	-20.1	0.6	2.1	
cattle	9	7.4	4.6	6.3	0.8	2.8	-13.5	-21.2	-18.4	2.4	7.7	
pig	9	8.1	5.6	7.0	0.8	2.5	-19.3	-20.4	-19.8	0.4	1.1	
dog	2	10	9	9.5	0.7	1.0	-18.5	-19.1	-18.8	0.4	0.6	
fox	3	7.6	4	6.4	2.1	3.6	-18.9	-22.5	-20.3	2.0	3.6	
hare	7	6.5	2.8	4.5	1.3	3.7	-20.7	-21.9	-21.3	0.5	1.2	
roe deer	2	6.5	6.4	6.5	0.1	0.1	-18.6	-19.9	-19.3	0.9	1.3	
fish	2	7.1	6.3	6.7	0.6	0.8	-13.0	-13.3	-13.2	0.2	0.3	
human	7	10.3	7.3	8.7	1.2	3.0	-19.0	-20.4	-20.0	0.5	1.4	

**Table 4** Summary statistics for all new plant and animal  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values from Halai presented in this study, divided into the five phases of Neolithic occupation of the site. SD refers to standard deviation.

species	n =	$\delta^{15}\text{N}$ max	$\delta^{15}\text{N}$ min	$\delta^{15}\text{N}$ average	$\delta^{15}\text{N}$ SD	$\delta^{15}\text{N}$ range	$\delta^{13}\text{C}$ max	$\delta^{13}\text{C}$ min	$\delta^{13}\text{C}$ average	$\delta^{13}\text{C}$ SD	$\delta^{13}\text{C}$ range
<b>A: Early Late Neolithic</b>											
hulled barley	2	2.6	2.2	2.4	0.3	0.4	-24.7	-24.9	-24.8	0.1	0.2
lentil	9	-2.2	-3.1	-2.6	0.3	0.9	-22.8	-25.7	-24.0	0.9	2.9
einkorn	1	1.2	1.2	1.2	-	0.0	-24.3	-24.3	-24.3	-	0.0
cattle	2	7.1	6.0	6.6	0.8	1.1	-13.5	-16.1	-14.8	1.8	2.6
pig	1	6.8	6.8	6.8	-	0.0	-19.5	-19.5	-19.5	-	0.0
<b>B: Early Middle Neolithic</b>											
hulled barley	12	5.5	-0.4	3.0	1.8	5.9	-23.4	-26.1	-24.4	0.7	2.7
free-threshing wheat	2	2.5	2.4	2.5	0.1	0.1	-23.5	-24.0	-23.8	0.4	0.6
einkorn	4	4.5	-1.2	2.3	2.6	5.7	-23.5	-26.0	-24.5	1.1	2.5
emmer	1	6.4	6.4	6.4	-	0.0	-24.0	-24.0	-24.0	-	0.0
lentil	11	1.7	-3.5	-1.2	2.0	5.2	-22.3	-25.1	-23.8	0.8	2.8
pea	5	1.8	-3.2	0.2	1.9	5.0	-23.7	-25.6	-24.6	0.7	1.9
sheep	5	6.9	4.5	5.6	0.9	2.4	-19.3	-20.8	-20.1	0.6	1.5
goat	4	6.5	3.0	5.5	1.6	3.5	-18.7	-20.6	-20.1	0.9	1.9
cattle	2	7.4	6.3	6.9	0.8	1.1	-17.1	-18.3	-17.7	0.8	1.2
pig	1	8.0	8.0	8.0	-	0.0	-19.6	-19.6	-19.6	-	0.0
<b>C: Late Middle Neolithic</b>											
hulled barley	7	5.6	0.2	3.7	1.7	5.4	-22.0	-24.7	-23.8	1.0	2.5
free-threshing wheat	2	2.2	0.2	1.2	1.4	2.0	-23.6	-24.9	-24.3	0.9	1.3



einkorn	3	-0.3	-1.7	-0.9	0.7	1.4	-23.1	-	25.0	-24.0	1.0	1.
oat	2	7.0	2.9	5.0	2.9	4.1	-24.7	-	25.0	-24.9	0.2	0.
lentil	8	1.4	-3.1	-1.0	2.0	4.5	-22.8	-	26.1	-24.0	1.0	3.
pea	2	-1.1	-2.7	-1.9	1.1	1.6	-23.9	-	24.2	-24.1	0.2	0.
grass pea	1	2.6	2.6	2.6	-	0.0	-24.6	-	24.6	-24.6	-	0.
sheep	3	6.3	5.8	6.1	0.3	0.5	-18.7	-	20.7	-19.7	1.0	2.
goat	3	6.0	5.1	5.5	0.5	0.9	-19.7	-	20.8	-20.3	0.6	1.
cattle	1	6.5	6.5	6.5	-	0.0	-19.9	-	19.9	-19.9	-	0.
pig	3	7.3	6.4	7.0	0.5	0.9	-19.3	-	19.7	-19.5	0.2	0.
dog	1	10.0	10.0	10.0	-	0.0	-18.5	-	18.5	-18.5	-	0.
fox	2	7.5	4.0	5.8	2.5	3.5	-18.9	-	22.5	-20.7	2.5	3.
hare	3	6.5	3.1	4.8	1.7	3.4	-20.7	-	21.4	-21.1	0.4	0.
human	4	9.3	7.3	7.9	0.9	2.0	-19.9	-	20.4	-20.2	0.2	0.
D: Early Late Neolithic												
hulled barley	7	5.6	-1.1	2.1	2.2	6.7	-22.5	-	25.1	-24.0	0.8	2.
einkorn	3	0.4	-0.6	-0.3	0.6	1.0	-22.0	-	23.9	-23.2	1.0	1.
oat	2	6.6	2.5	4.6	2.9	4.1	-24.4	-	24.7	-24.6	0.2	0.
lentil	1	-3.4	-3.4	-3.4	-	0.0	-24.9	-	24.9	-24.9	-	0.
pea	1	-3.1	-3.1	-3.1	-	0	-26	-	-26	-25.9	-	0.
sheep	9	8.2	3.4	5.9	1.4	4.8	-16.2	-	21.3	-19.7	1.5	5.
goat	6	6.4	5.5	5.8	0.4	0.9	-19.6	-	20.4	-20.0	0.4	0.
cattle	3	6.6	5.7	6.2	0.5	0.9	-18.8	-	20.4	-19.7	0.8	1.
pig	3	7.2	5.6	6.4	0.8	1.6	-20.0	-	20.4	-20.2	0.2	0.

dog	1	9.0	9.0	9.0	-	0.0	-19.1	-	19.1	-19.1	-	0.
fox	1	7.6	7.6	7.6	-	0.0	-19.4	-	19.4	-19.4	-	0.
hare	3	5.1	4.0	4.7	0.6	1.1	-20.7	-	21.9	-21.4	0.6	1.
roe deer	2	6.5	6.4	6.5	0.1	0.1	-18.6	-	19.9	-19.3	0.9	1.
fish	2	7.1	6.3	6.7	0.6	0.8	-13.0	-	13.3	-13.2	0.2	0.
human	1	10.3	10.3	10.3	-	0.0	-19.0	-	19.0	-19.0	-	0.
E: Late Late Neolithic												
hulled barley	1	4.0	4.0	4.0	-	0.0	-24.0	-	24.0	-24.0	-	0.
einkorn	2	2.0	-0.3	0.9	1.6	2.3	-23.7	-	24.4	-24.1	0.5	0.
lentil	1	-2.2	-2.2	-2.2	-	0.0	-26.1	-	26.1	-26.1	-	0.
pea	1	1.0	1.0	1.0	-	0.0	-26.8	-	26.8	-26.8	-	0.
sheep	2	6.2	6.0	6.1	0.1	0.2	-19.8	-	20.0	-19.9	0.1	0.
goat	1	5.7	5.7	5.7	-	0.0	-19.7	-	19.7	-19.7	-	0.
cattle	1	4.6	4.6	4.6	-	0.0	-21.2	-	21.2	-21.2	-	0.
pig	1	8.1	8.1	8.1	-	0.0	-19.9	-	19.9	-19.9	-	0.
hare	1	2.8	2.8	2.8	-	0.0	-21.7	-	21.7	-21.7	-	0.
human	2	9.6	9.3	9.5	0.2	0.3	-20.1	-	20.2	-20.2	0.1	0.

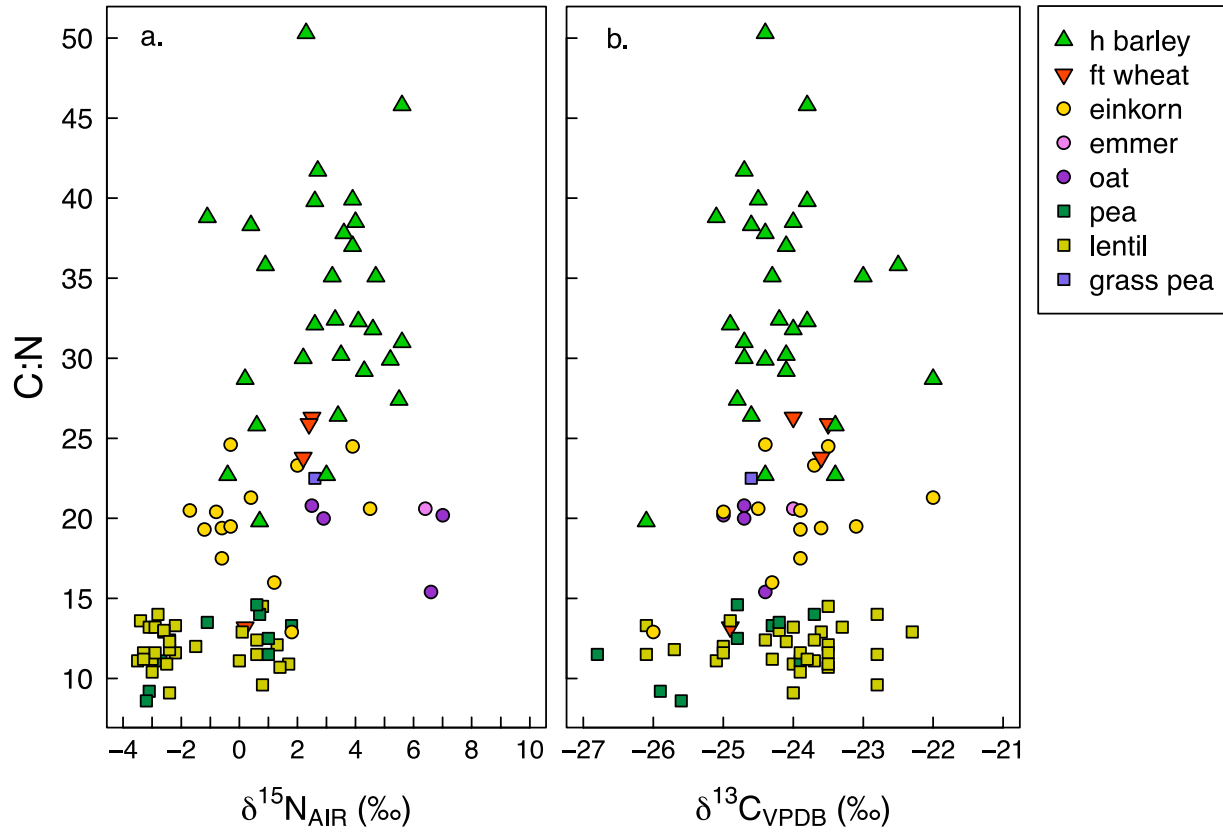
**Table 5** Results of multi-variate analyses carried out on the stable isotopic results from Halai. Normality of data was tested using Shapiro-Wilk test (null hypothesis: data is not normally distributed). Homogeneity of data was tested using Levene's test (null hypothesis: data is not homogeneous).

	Shapiro-Wilk test	normality	Levene's test	homogeneity	mult
<b>hulled barley</b>					
$\delta^{15}\text{N}$	W = 0.946, p = 0.147	normal	F(4,24) = 1.082, p = 0.387	homogeneous	
$\Delta^{13}\text{C}$	W = 0.941, p = 0.104	normal	F(4,24) = 0.884, p = 0.488	homogeneous	
<b>lentil</b>					
$\delta^{15}\text{N}$	W = 0.811, p < 0.001	not normal			Kru
$\Delta^{13}\text{C}$	W = 0.946, p = 0.134	normal	F(4,25) = 0.504, p = 0.733	homogeneous	
<b>einkorn</b>					
$\delta^{15}\text{N}$	W = 0.896, p = 0.116	normal	F(4,8) = 2.531, p = 0.123	homogeneous	
$\Delta^{13}\text{C}$	W = 0.957, p = 0.713	normal	F(4,8) = 0.679, p = 0.626	homogeneous	

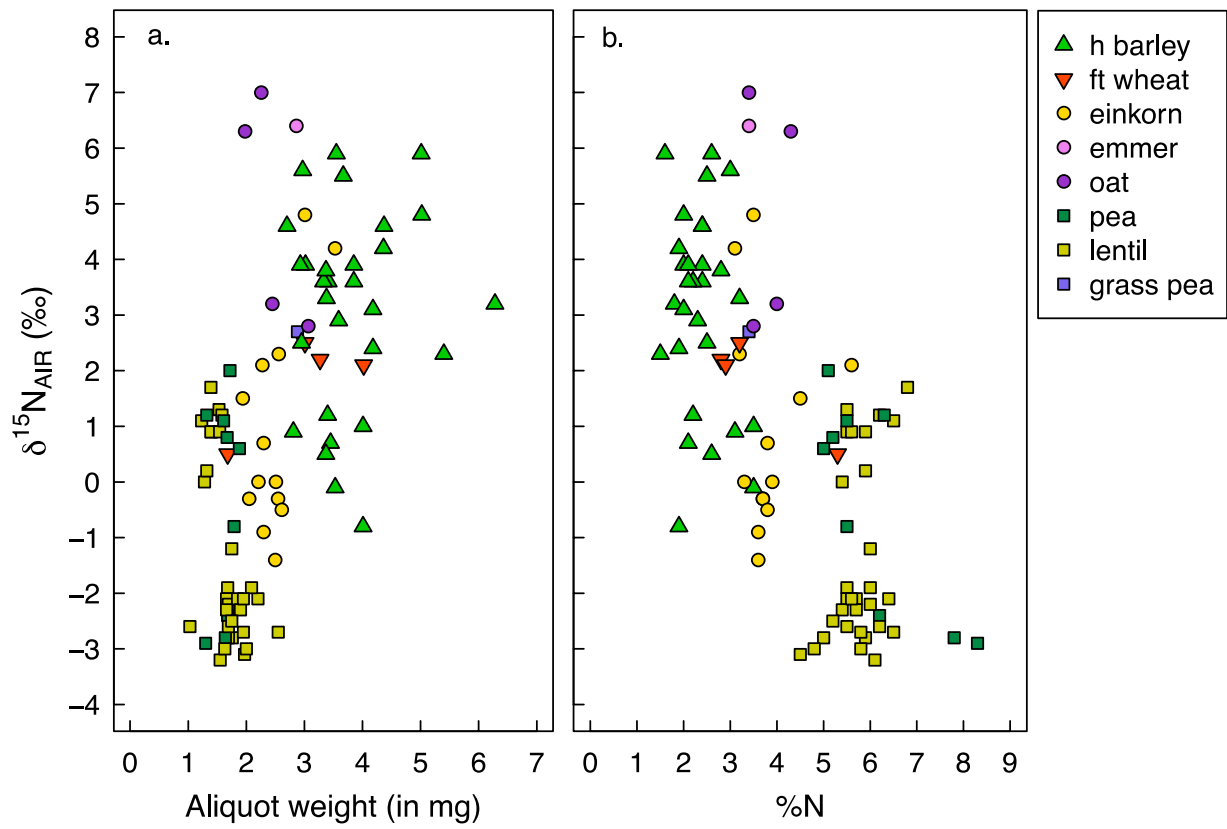
**Table 6** Summary statistics for all new and previously published plant and animal  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values from Makriyalos II. See Table 2 for reference for previously published data. SD refers to standard deviation.

species	n =	$\delta^{15}\text{N}$ max	$\delta^{15}\text{N}$ min	$\delta^{15}\text{N}$ average	$\delta^{15}\text{N}$ SD	$\delta^{15}\text{N}$ range	$\delta^{13}\text{C}$ max	$\delta^{13}\text{C}$ min	$\delta^{13}\text{C}$ average	$\delta^{13}\text{C}$ SD	$\delta^{13}\text{C}$ range	$\Delta^{13}\text{C}$ max
lentil	3	3.2	1.2	1.9	1.1	2.1	-22.7	-25.3	-23.7	1.4	2.6	19.3
grass pea	1	1.6	1.6	1.6	-	0.0	-25.5	-25.5	-25.5	-	0.0	19.5
terebinth	1	1.8	1.8	1.8	-	0.0	-23.5	-23.5	-23.5	-	0.0	17.4
sheep	5	6.1	3.6	4.9	0.9	2.4	-18.7	-21.0	-19.8	0.9	2.3	-
goat	5	4.3	2.4	3.3	0.7	1.9	-19.1	-20.9	-20.1	0.7	1.7	-
cattle	4	4.7	3.6	4.4	0.5	1.2	-14.7	-20.4	-18.1	2.4	5.7	-
red deer	9	6.1	1.1	3.5	1.4	5.0	-21.1	-22.2	-21.7	0.3	1.1	-
roe deer	2	5.9	3.4	4.6	1.8	2.5	-21.2	-21.8	-21.5	0.5	0.7	-
pig	5	6.2	4.4	5.3	0.8	1.8	-20.2	-20.9	-20.5	0.3	0.6	-
boar	5	5.0	2.8	4.2	0.8	2.2	-20.0	-21.0	-20.4	0.4	1.0	-

## Supplementary Figures



**Supplementary Figure S2.** Atomic C:N ratios of charred plants analyzed in this study vs. (a)  $\delta^{15}\text{N}$  values and (b)  $\delta^{13}\text{C}$  values.



**Supplementary Figure S3.** Charred plant  $\delta^{15}\text{N}$  values measured in this study vs. (a) aliquot weight (in mg), and (b) nitrogen content (%N).