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## **Specifying subsistence strategies of Early farmers: new results from compound specific isotopic analysis of amino acids (CSIA-AA)**

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**KEYWORDS:** Neolithic, bone collagen, nitrogen stable isotope, freshwater resources, manured Cereals

### **Running head: Early farmers' subsistence by using isotopic analysis of amino acids**

### **ABSTRACT:**

Understanding the contribution of fish to the human diet is an important debate in past population studies as it implies specific organization (e.g. adapted techniques, gender-based activities?) and relationship with the local or sublocal environments (e.g. mobility, seasonal-based exploitation). In the Paris Basin, the role of fish has been discussed mainly from rare but obvious archaeological remains such as fish bones or hooks. Previous isotopic data from Neolithic human bone bulk collagen highlighted the potential of freshwater resources in protein diet but were not able to quantify it or even confirm its role. In this study we conducted, for the first time on early farmers from France, compound specific isotope analysis on bone collagen amino acids (CSIA-AA). Results indicate a particularly high trophic level of some humans with protein intake mainly based on terrestrial animal resources (domestic cattle and pig), while others rather have a plant-based diet. Freshwater resources appeared to be not significant and several possible food combinations are discussed. Pig particularly appears more herbivorous than previously assumed and has possibly consumed manured cereals from human food waste.

## 1. INTRODUCTION

The Neolithic period represents a major change in people's lifestyles, mainly due to the transition from a hunter-gatherer economy to an economy of production (husbandry and agriculture). Humans were settling down, establishing villages, storing their food and structuring themselves with the emergence of increased hierarchies and prestige (e.g. Beyneix, 2016; Cassidy et al., 2020; Jeunesse, 2016; Thomas, 2014). The study of food allows an approach to these new social practices. For example, some French studies suggest that hunting was becoming more symbolic, as hunted animals took the form of prestigious objects (Jeunesse and Arbogast, 1997; Sidéra, 2003). However, this assumption is debated, as broader-spectrum feeding societies (based on both farming and hunting/gathering/fishing) could be more egalitarian, while the production and storage of food could represent social competition in a "banquet economy" (Jeunesse, 2010). The choice of food resource management (collecting wild foods, including aquatic foods vs. producing and storing meat, dairy and cereals, and in which proportions) raises the question of economic and social organization. This gives an insight of the breadth of the topic and the importance of the role of food in our human societies, past and present.

In the Middle Neolithic period, in the North and Centre of France, humans consumed cattle, to a lesser extent pigs and finally caprines (sheep and goat; e.g. Arbogast et al., 2018; Bedault, 2009; Hachem et al., 2014). Dairy products are also evidenced, but in unknown proportions (Balasse and Tresset, 2002; Drieu et al., 2020; Gillis et al., 2017). The contribution of plants is difficult to quantify as there are few preserved remains, but cereals (wheat and barley) are well attested (Bakels, 1997; Hamon et al., 2019; Martin et al., 2014; Meunier et al., 2006) – whereas millet (C4 plant) appeared later in northern France during the Bronze Age (Auxiette et al., 2018; Goude et al., 2017). In this respect, manuring practice is already proposed for some European sites (Bogaard et al., 2013; Fraser et al., 2013; Gron et al., 2017) and should be considered in France. Furthermore, remains of wild plants, peas, lentils, hazelnuts, etc. have also been retrieved in northern France, though their consumption is more difficult to demonstrate (Bouby et al., 2018; Dietsch-Sellami, 2007; Marinval, 1995). Overall, recent isotopic studies have shown that the proportion of plants in the diet is often underestimated in the European Neolithic context (Bickle, 2018; Bogaard et al., 2017; Styring et al., 2018).

Moreover, aquatic resources could also have been consumed in the Neolithic period as a complement to terrestrial supplies. While some studies have demonstrated the abandonment of marine resources at the onset of the Neolithic in marine coastal areas (Richards et al., 2003), the picture is more complex than it appears depending on the region (Bickle, 2018; Salazar-García et al., 2018), several isotopic studies have shown a drop but still a continuity of freshwater fish consumption in European inland sites, as for example in the Danube Gorges (de Beccdelièvre et al., 2020; Jovanović et al., 2015), in Belgium (Bocherens et al., 2007), in Ukraine (Budd et al., 2020; Lillie et al., 2011), in the Baltic (Lithuania; Piličiauskas et al., 2017) and in Germany (Bickle, 2018; Fernandes et al., 2015). In Northern France, fishing is well attested in the Mesolithic period at Noyen-sur-Seine and La Chaussée-Tirancourt (Drucker et al., 2018), but it is less obvious for the Neolithic period. Some sites in the Paris Basin have yielded several remains of cyprinidae, pike, perch and eel (Clavel, 2009; Clavel and Arbogast, 2007). Moreover, a hook has been discovered in a grave in the Yonne Valley, in the site of Monéteau (close to the sites of Gurgy and Beaumont studied here; Augereau and Chambon, 2011, p.311; Augereau et al., 2005). Though such regional evidence is scarce, freshwater fish consumption should be considered.

In the North and Center of France, previous isotopic studies of bulk bone collagen have revealed high nitrogen isotopic ratios in humans compared to domestic animals. At Gurgy "Les Noisats" (GLN) and Pontcharaud (NFN; Fig. 1), carbon (C), nitrogen (N) and sulfur (S) isotopic ratios all suggest a mixed consumption of herbivores (mainly cattle, in agreement with zooarchaeological data) and pigs, with

possible addition of freshwater fish (Fig. 2; Goude et al., 2013; Rey et al., 2019). Terrestrial wild animals do not seem to have been consumed to a substantial extent, in accordance with the zooarchaeological data which suggests that hunting was a rather symbolic activity at this period. The role of plants in the diet is not known and the trophic level (TL) of humans given by the nitrogen enrichment between humans and domestic animals is particularly high with respect to what is known in other contemporaneous sites ( $\Delta^{15}\text{N}_{\text{animals-humans}} = 4.7\text{\textperthousand}$  at GLN,  $\Delta^{15}\text{N}_{\text{animals-humans}} = 3.8\text{\textperthousand}$  at NFN and, in average,  $\Delta^{15}\text{N}_{\text{animals-humans}} = 2.9 \pm 1\text{\textperthousand}$  for about thirty Neolithic neighboring sites; Rey et al., 2019 Online Resource 10 and 11). In comparison, such a high value of  $\Delta^{15}\text{N}_{\text{animals-humans}}$  has been observed at the Les Bréguières site (4.5‰) and Baume-Bourbon (4.5‰) in the South of France, where the heavy reliance on terrestrial animal protein, with possible consumption of aquatic food, was proposed (Goude, 2007; Goude et al., 2020). The role of plants, of lower trophic level and poorer protein content, is all the more hidden in bulk collagen when other resources of high trophic level and richer in protein are consumed, like animal resources and especially fish. For GLN and NFN, several hypotheses, among various possibilities, have been proposed without being able to reach a conclusion: the human diet could be particularly rich in animal proteins (stable isotope analysis of bulk bone collagen cannot distinguish between meat and milk proteins for example); cereals could be manured leading to an increase in  $\delta^{15}\text{N}$  and thus an underestimation of their proportion in diet (plants consumed by humans having different values than forage, as suggested by A. Bogaard and colleagues (2017; 2013); freshwater fish, supposed to have higher  $\delta^{15}\text{N}$  values than terrestrial animals, could be part of the diet in addition to domestic animals. As neither plant remains nor fish could be retrieved on the sites and analyzed, it is challenging to estimate the position of humans in their local food chain. Without known values of plants, at the bottom of the food chain, and fish at the top, the “intermediate” values of humans can be explained by a multitude of interrelated factors. In addition, the  $\Delta^{15}\text{N}_{\text{diet-bulk collagen}}$  offset can vary generally between 3 and 5‰ (Bocherens and Drucker, 2003), up to 6‰ according to some authors (O’Connell et al., 2012).

While S isotope analysis can be useful to evaluate freshwater fish consumption in some contexts (e.g. (Nehlich et al., 2010)), the previous dataset from Northern France sites remains inconclusive: human values overlap with both cattle and pig values (which are distinct), and it is not known whether local freshwater fish values could be distinct from this terrestrial range (Fig. 2; Rey et al., 2019). A survey of the literature showed the wide variation of freshwater C, N and S isotopic ratios with environmental conditions and the possible overlapping between aquatic and terrestrial ecosystems. The isotopic variability is illustrated in Appendix Fig. A1 for various French, Belgian and German sites (see also Dufour et al., 1999; Dufour and Gerdeaux, 2001; France, 1994 for a general purpose). The large variation of plant values is also demonstrated (Appendix Fig. A1 for a nearby regional context in the Neolithic; see also Gavériaux et al., accepted for Neolithic Southern France). Notwithstanding, the inverse correlation observed between the  $\delta^{34}\text{S}$  and  $\delta^{15}\text{N}$  values of human bulk collagen from GLN possibly supports the complementary consumption of freshwater resources, as suggested by (Nehlich et al., 2014) for the German Neolithic site of Großstorkwitz, although it could also reflect the relative reliance on food from two distinct locations. On another note, no depleted  $^{14}\text{C}$  values in bulk collagen which could reflect any reservoir effect were identified, giving no evidence for or against the consumption of freshwater resources (as many aquatic systems do not have any offset from atmospheric  $^{14}\text{C}$  levels ; Cook et al., 2001; Philippson and Heinemeier, 2013).

In this context, the CNS stable isotope analysis on bulk collagen remained very limited to clearly assess the possible contribution of freshwater fish in the diet and consequently also to estimate the relative contribution of plants. Consequently, our study proposes to estimate the trophic position (TP) of individuals without requiring *a priori* any local baseline (as food source values) by using compound specific isotopic analysis of amino acids (CSIA-AA; Chikaraishi et al., 2010a).

## 2. MATERIALS AND METHOD

### 2.1 Brief description of the sites

The samples come from four Neolithic sites located in three French regions (Fig. 1). Beaumont “Le Crot aux Moines” (BMT; 4800-4000 cal BC) is an enclosure located in the Yonne Valley (Paris Basin) and attributed to the Chasséen culture. It contains scattered remains of humans and animals conjointly (Chambon et al., 2009). This site has only provided two animal samples for baseline. Gurgy “Les Noisats” (GLN; 5200-3800 cal BC), also located in the Yonne Valley, is one of the biggest cemeteries for the French Neolithic containing more than 120 burials, mainly individuals (Rivollat et al., 2015; Rottier, 2007; Rottier et al., 2005). The few but highly diversified grave goods did not allow a strict cultural attribution but refer to several cultures of the Early and Middle I Neolithic. Very few faunal remains have been retrieved and they are mostly used for ornaments or tools. Most of the samples analyzed in this study (6 humans and 3 animals) came from this site. Le Vigneau 2 (LV; 4720-4350 cal BC) is a large cemetery of 102 mostly individual burials located in Pussigny (Loire Valley; Coutelas et al., 2015). The graves are dated to the Middle Neolithic and belong to the Chambon culture. The buried people were probably shepherds. Ovis remains are repeatedly found with the human skeletons as funerary offerings. From this site, one animal collagen sample is tested by CSIA-AA (LVF9; sheep) (see also Goude et al., 2019). Pontcharaud 2 (NFN; 4460-4030 BC cal), located in Clermont-Ferrand (Auvergne), is a more recent funerary ensemble composed of 58 mainly individual burials (Loison, 1998). This large ensemble belongs to the Middle I Neolithic of the Limagne. For this site as for GLN, the hypothesis of freshwater fish consumption by the humans has been proposed on the basis of bulk collagen isotopic data (Goude et al., 2013). The collagen of one animal (NFN A01; cattle) and one adult female (NFN28) are tested by CSIA-AA. The few samples from these two contemporaneous sites will (1) help to document the geographic variability, (2) provide further investigation about  $\delta^{15}\text{N}_{\text{bulk}}$  and TPs comparison to understand the specificity of the Neolithic dietary schemes (for which domestic resources are supposed to be dominant) and (3) allow, for the first time in farmers from western Europe, to evaluate the potential of CSIA-AA to re-discuss the variability of dietary pattern in communities with possible different social organization.

### 2.2 Sample selection

In total, the analyses were performed on 7 humans (4 adult females, 2 adult males, 1 immature female), 6 herbivores (2 cattle, 2 sheep/goat, 2 deer) and 1 pig, omnivorous (Table 1). The main archaeological and analytical data are summarized in Table 1 and further details are available in corresponding references. The samples come from previously prepared bulk bone collagen for stable C, N and S isotope analysis. Results of these analyses are available in previous studies (Goude et al., 2021; Goude et al., 2019; Goude et al., 2013; Rey et al., 2017; Rey et al., 2019) and are summarized in Table 2. All the samples were chosen for the good preservation of the remaining collagen available and, when possible, to get the best representation of the isotopic variability according to their C, N and S values (see the distribution of the selected samples’ isotopic values among the overall distribution of each site for the three regions studied in Appendix Fig. A2). We are aware that such a small number of samples limits the representativeness of the human group variability but it provides the first opportunity to test this methodology on Early European farmers.

### 2.3 $\delta^{15}\text{N}_{\text{AA}}$ measurements

All the collagen samples were previously extracted and their quality was certified (see corresponding references). The amino acid samples were prepared following the JAMSTEC established protocol (see Naito et al., 2013b for more details). Collagen was hydrolyzed in 12N HCl at 110°C for 12h. Then, derivatization was carried out with thionyl chloride/2-propanol (TC/iPr, 1/4, v/v) at 110°C for 2h (isopropyl esterification) and with pivaloyl chloride/dichloromethane (PC/DCM, 1/4, v/v) at 110°C for 2h (acylation). The organic solvent layer was extracted and dehydrated. After amino acids were verified by GC-FID/NPD, the nitrogen isotopic composition of individual amino acids was measured by GC-C-IRMS (gas chromatography-combustion-isotope ratio mass spectrometry) using an Agilent Technology 6890 GC coupled to a ThermoFinniganDeltaplus XP IRMS via combustion and reduction furnaces. Instrumental analysis was performed according to Chikaraishi et al. (2007) and Chikaraishi et al. (2010b). The analytical error was <0.5‰.

#### 2.4 Trophic position (TP) estimation

Assessing the TP of samples relies on the following principles: 1) the  $\delta^{15}\text{N}$  of glutamic acid ( $\delta^{15}\text{N}_{\text{Glu}}$ ) greatly increases from prey to consumer ( $+8\text{\textperthousand} \pm 1.1\text{\textperthousand}$ ) while the  $\delta^{15}\text{N}$  of phenylalanine ( $\delta^{15}\text{N}_{\text{Phe}}$ ) varies little ( $+0.4 \pm 0.4\text{\textperthousand}$ ) in both aquatic (Chikaraishi et al., 2007; Chikaraishi et al., 2009; McClelland and Montoya, 2002) and terrestrial (Chikaraishi et al., 2011) ecosystems – this implies that  $\delta^{15}\text{N}_{\text{Glu}}$  rather reflects the trophic position (it increases from plants to herbivores and then to carnivores; e.g. Ohkouchi et al., 2017; Itahashi et al., 2019) while  $\delta^{15}\text{N}_{\text{Phe}}$  rather reflects the source at the bottom of the food chain, namely the environment, and can be particularly useful to distinguish freshwater fish, whose  $\delta^{15}\text{N}_{\text{Phe}}$  is often lower than in terrestrial animals (e.g. Naito et al., 2013a; Itahashi et al., 2019; 2020); 2) the  $\Delta^{15}\text{N}_{\text{Glu-Phe}}$  value ( $\delta^{15}\text{N}_{\text{Glu}} - \delta^{15}\text{N}_{\text{Phe}}$ ) of primary producers, also called  $\beta$  factor, distinguishes between C<sub>3</sub> terrestrial plants ( $\beta \approx -8.4\text{\textperthousand}$ , although this  $\beta$  factor is potentially variable and this will be discussed later) and aquatic plants such as algae and cyanobacteria ( $\beta \approx +3.4\text{\textperthousand}$ ; Chikaraishi et al., 2007; Chikaraishi et al., 2010a; Fig. 3). According to these observations, the TP of samples can be estimated by the following equations:

$$\text{TP}_{\text{AA}}(\text{C}_3) = ([\delta^{15}\text{N}_{\text{Glu}} - \delta^{15}\text{N}_{\text{Phe}} + 8.4] / 7.6) + 1 \text{ for terrestrial C}_3 \text{ plants-based ecosystems (1)}$$

$$\text{TP}_{\text{AA}}(\text{aqua}) = ([\delta^{15}\text{N}_{\text{Glu}} - \delta^{15}\text{N}_{\text{Phe}} - 3.4] / 7.6) + 1 \text{ for aquatic ecosystems (2)}$$

where  $\text{TP}_{\text{AA}}$  indicates the amino acid-based trophic position and the 7.6 value corresponds to the mean change in the Glu-Phe difference between trophic levels, called trophic discrimination factor or TDF (Chikaraishi et al., 2007; Chikaraishi et al., 2011). TP = 1, TP = 2 and TP = 3 indicate respectively primary producers (plants), primary consumers (herbivores) and secondary consumers (carnivores). In the following sections, TP is used for terrestrial TP, unless otherwise indicated. While the traditional isotopic analysis of collagen needs a local baseline to estimate the position of individuals into their trophic chain (due to large environmental and physiological variations of the values), these equations do not require in theory the characterization of the  $\delta^{15}\text{N}$  values at the bottom of the food chain to estimate the TP. They use the isotopic spacing between two amino acids from the same specimen, thus allowing direct comparisons of the  $\text{TP}_{\text{AA}}$  of individuals originating from various spatiotemporal contexts (Chikaraishi et al., 2014). However, in the case of mixed diet, it is helpful to measure local animal values to disentangle possible variations.

#### 2.5 Baseline references and estimations

To compare the human isotopic values with those of food items probably consumed, we have referenced all available data from contexts as close as possible to our sites. All the data is available in Appendix Table A1-7 and mean values or estimates of each food source considered are represented in Figure 2 and 4. The Yonne baseline inventories all the isotopic values previously obtained from

several Neolithic sites in a radius of 10 km in the Yonne valley including GLN and BMT. While the values of local and contemporary terrestrial animals were measured directly along with humans, no plant or fish remains were found on the sites. Archaeoichthyological and archaeobotanical data are besides very scarce in the region and the consumption/production/management of these resources is rather unknown. Consequently, the plants and freshwater baselines are taken from the literature (Appendix Table A4-6).

The baseline for plants is estimated from available data from German Neolithic sites (Styring et al., 2017). Mediterranean and further North sites have a relatively different climate and environment that may impact the isotopic ratios (Craine et al., 2009; Gavériaux et al., accepted; Goude and Fontugne, 2016). They have therefore not been considered as baseline here. Earlier sites do not yield cereals, recently cultivated by early farmers, and more recent sites may be more subject to more intense agricultural practices (intensive manuring and water management; e.g. Varalli et al., 2021), so they are also excluded here. As it has been shown that humans did not consume the same plants as herbivores (Bogaard et al., 2017), we preferred to consider, for C and N isotopes, plant values measured in other sites from a similar context (Neolithic Germany; Styring et al., 2017) rather than our local herbivore values subtracting the trophic enrichment (this gives the forage values). Contrarily, for S, plant values have been estimated from domestic fauna, as suggested by Fernandes et al. (2015), since S isotopic ratio is highly variable with environment (Nehlich, 2015) and there is no fractionation between prey and consumer (Nehlich, 2015; Tanz and Schmidt, 2010). For AA, we used the  $\delta^{15}\text{N}_{\text{Glu}}$  value estimated by Styring et al. (2015) for the German Neolithic site of Vaihingen. As archaeological plants do not contain sufficient AAs, the  $\delta^{15}\text{N}_{\text{Glu}}$  value is roughly estimated from comparison of their measured  $\delta^{15}\text{N}_{\text{bulk}}$  and empirically known difference between  $\delta^{15}\text{N}_{\text{bulk}}$  and  $\delta^{15}\text{N}_{\text{Glu}}$  in modern cereal grains. In this case, this means subtracting 0.5‰ from the  $\delta^{15}\text{N}_{\text{bulk}}$  values of charred grains from Vaihingen. In addition, the plant  $\delta^{15}\text{N}_{\text{Phe}}$  value is estimated from the average human and domestic animal  $\delta^{15}\text{N}_{\text{Phe}}$  value of the samples analyzed in this study minus the theoretical trophic enrichment of 0.4‰ (namely  $\delta^{15}\text{N}_{\text{Phe}} \approx 11.2 (\pm 1.7) - 0.4 \approx 10.8 \pm 1.7 \text{‰}$ ) according to Styring et al. (2015) recommendations. This value is the same as those estimated for Vaihingen (*ibid*).

For aquatic resources, namely freshwater fish (riparian are not considered in this study, since they have never been found in the region for the Neolithic period and they are not supposed to be consumed), we have considered data from as many sites as possible located in neighboring regions and belonging to similar or earlier periods (the latter to avoid any pollution effect occurring later).

### 3. RESULTS AND DISCUSSION

All the collagen samples were well-preserved according to commonly accepted criteria (all details were already published in references cited in Table 1). The analytical quality for CSIA-AA is assessed with standard calibration and through good resolution on GC chromatogram and reproducibility of measurements. All the results are given in Table 2 with calculated TPs and plotted on Figure 2 for bulk collagen and on Figure 4 for AA. Considering a constant  $\beta$  value of  $-8.4 \pm 0.2 \text{‰}$  (Chikaraishi et al., 2011), all domestic and wild herbivores have a TP of  $2 \pm 0.2$ , in agreement with expected values. Pig has a TP of  $2.1 \pm 0.2$  corresponding to a rather herbivorous diet. Humans have TPs from  $2.2$  to  $3.2 \pm 0.2$ , which corresponds to a diet ranging from almost herbivore to quite carnivore. Overall, the  $\delta^{15}\text{N}_{\text{Phe}}$  values of animals vary widely, as commonly observed (Ohkouchi et al., 2017). The  $\delta^{15}\text{N}_{\text{Phe}}$  values of wild deer samples are on average lower than those of domestic species. There is no apparent difference of  $\delta^{15}\text{N}_{\text{Phe}}$  values between sites. Humans have similar  $\delta^{15}\text{N}_{\text{Phe}}$  values to domestic animals.

The results illustrated in Figure 4 show that herbivores' values are well aligned along the line  $TP_{ter} = 2.0 \pm 0.2$  corresponding to a pure herbivorous diet, in agreement with expected values. This confirms the reliability of the measurements. The apparent variability in  $\delta^{15}\text{N}_{\text{Phe}}$  values between wild and domestic species could reflect different occupied environment. Livestock could be kept close to the settlements while deer were roaming free further, probably in a more covered environment, as suggested also by lower C and N isotopic values of bulk collagen than those of domestic species. Moreover, the pig's  $\delta^{15}\text{N}_{\text{Phe}}$  value is slightly higher than those of herbivores (see below). Despite the limited number of samples, the difference in  $\delta^{15}\text{N}_{\text{Phe}}$  between wild herbivores, domestic herbivores and pig fits with the differences observed in bulk collagen for C, N and S isotopic ratios (Fig. 2; Rey et al., 2019). This is in line with the already proposed hypothesis of keeping the different species in separate spaces and/or feeding them differently (*ibid*).

On the contrary, the Neolithic humans' results show a wide variability of the TPs, while the range of  $\delta^{15}\text{N}_{\text{Phe}}$  values is similar to that of animals, especially when excluding the roe deer (Fig. 4). On the one hand, this further supports the previous result on bulk collagen: great variability for N isotope ratios (reflecting trophic effect). On another hand, while previous C and to a lesser extent S isotope ratios (rather linked to the source and environment) were rather homogeneous when compared to the animals (Fig. 2; Rey et al., 2019), here the  $\delta^{15}\text{N}_{\text{Phe}}$  values appeared slightly more variable. The human  $\delta^{15}\text{N}_{\text{AA}}$  results indicate a varied diet based on domestic plants and animals in extremely different proportions between individuals, with probably no significant freshwater resources (see details later). No sex-related distinction is highlighted (Fig. 4), contrarily to what has been observed for C, N and S isotope ratios on bulk collagen in the whole GLN sample (Rey et al., 2019). However, here the sample size is too small and unbalanced to draw conclusions. Regarding the animal component, the results are consistent with the mixed consumption of cattle and pig, agreeing with previous isotopic and zooarchaeological data. However, the proportion of animal intake varies greatly between individuals.

First, the lowest TP is obtained for GLN 284 ( $TP \approx 2.2$ ), which most likely had a very predominantly plant-based diet. This supports previous result for bulk collagen, as the  $\delta^{15}\text{N}_{\text{bulk}}$  value is significantly lower than all the other adults from the same site. Although zooarchaeological data suggest that wild animals were little consumed by these people, the extreme  $\delta^{15}\text{N}_{\text{bulk}}$  and  $\delta^{34}\text{S}_{\text{bulk}}$  values of this woman (Fig. 2) could suggest that she may have been an exception to the rule. However, the AA results definitely exclude this possibility as her low trophic level most likely reflects a mainly plant-based diet (Fig. 4). This woman could have had a particular social status with specific diet or could not eat meat for physiological reason (intolerance, allergy, etc.). The hypothesis of a disturbed health condition leading to a nutritional stress and  $^{15}\text{N}$ -depleted ratio has also been proposed (e.g. Hatch et al., 2006; Petzke et al., 2006). Unfortunately, the preservation state of the remains does not allow us to investigate this issue. However, due to the slow turnover of bone collagen, only a long term physiological disorder might significantly alter the  $\delta^{15}\text{N}$  (Katzenberg and Lovell, 1999). Similarly, while pregnancy can vary the  $\delta^{15}\text{N}$  values in different ways throughout gestation (e.g. D'Ortenzio et al., 2015; Fuller et al., 2005), it is unlikely that a succession of pregnancies could have caused a variation that would be visible to this extent in the bone, which registers an averaged signal over several years (e.g. Valentin, 2002).

On the other hand, the sample NFN 28 has an intermediate TP ( $\approx 2.4$ ) rather reflecting an omnivorous diet with a large proportion of plants. This result supports no freshwater fish consumption. At the site level, although other individuals presented higher  $\delta^{15}\text{N}_{\text{bulk}}$  value than NFN 28 (Fig. 2), the latter's value is close to the average of all the adults (mean  $\delta^{15}\text{N}_{\text{bulk}} = 10.3 \pm 0.8\%$ ; Goude et al., 2013). It is thus reasonable to assume that for most of the humans of this site, fish was not part of the staple diet.

Its consumption is however not excluded occasionally for a few humans with the highest  $\delta^{15}\text{N}_{\text{bulk}}$  values of this group (up to 13 ‰).

Finally, all the other individuals from GLN have a high TP from approximately 2.7 to 3.2. In terrestrial environments, this reflects a highly carnivorous diet, based on almost 100% of herbivore meat or milk for  $\text{TP} \approx 3$ , which is not expected for a French Neolithic population. However, the mixed consumption of resources of different trophic levels could also lead to this result. This is particularly so when the diet includes omnivorous or carnivorous species or fish in addition to herbivore products and plants, as it is suspected here on the basis of C, N and S isotope ratios of bulk collagen. Consequently, we propose to examine different food combinations.

The first food item considered is pig, for which regional zooarchaeological data indicates that it was the second animal resource consumed after cattle (Arbogast et al., 2018; Bedault, 2009; Hachem et al., 2014). While pigs from GLN were supposed to be omnivorous according to their  $\delta^{15}\text{N}_{\text{bulk}}$  values which are higher than those of herbivores (Fig. 2), here the only sample analyzed has a TP close to herbivores ( $\approx 2.1$ ). This result could reflect a much more plant-based diet than expected. In this case, the high TP of humans is all the more surprising, indicating an almost exclusive/very largely predominant consumption of animal products (when excluding aquatic resources intake). Although pigs can be significantly fed on human food waste, here the difference between the two species in C, N and S isotope ratios of bulk collagen indicates that they do not have the same protein intake (Fig. 2), as also suggested by the CSIA-AA results for nitrogen. Nevertheless, if their diet was more omnivorous than the AA isotope result indicates, it is possible that pigs have consumed lower TP plants. Indeed,  $\beta$  factor is variable and some authors have shown that trees in particular have lower values than herbaceous plants (Kendall et al., 2019; see Appendix Fig. A1.f). The consumption of oak acorns, for example, could decrease the TP of pigs, in addition to animal resources. Indeed, pig is known to be an omnivorous, burrowing animal, feeding on plants, tubers and roots, but also on small animals (insects, mollusks, small mammals or others). The  $\beta$  factor variability has been highlighted by several studies, either for plant species (e.g. cereals vs. legumes) or anatomical parts (e.g. rachis vs. grain), allowing various possibilities regarding the plant consumption impact on the pigs' TP (see Appendix Fig. A1.f; Bol et al., 2002; Itahashi et al., 2019; Kendall et al., 2019; Paolini et al., 2015; Steffan et al., 2013; Styring et al., 2014b; Takizawa et al., 2017; Yoneyama and Tanaka, 1999). Moreover, TDF has also been reported to be variable, adding uncertainty to our interpretations, although it is mostly referred to be between 7 and 8 for terrestrial mammals (e.g. Chikaraishi et al., 2015; Kendall et al., 2017; McMahon and McCarthy, 2016).

In farming conditions, pigs can be fed essentially with plants, in particular with cereal crop waste, possibly including manured crops which present higher  $\delta^{15}\text{N}$  values. This hypothesis is consistent with the observed  $\delta^{15}\text{N}_{\text{AA}}$  results (Fig. 4): herbivore's TP and both higher  $\delta^{15}\text{N}_{\text{Glu}}$  and  $\delta^{15}\text{N}_{\text{Phe}}$  values than other domestic samples. Indeed, manuring leads to an increase of all the  $\delta^{15}\text{N}_{\text{AA}}$  to a same extent resulting in an unchanged  $\text{TP}_{\text{AA}}$  (Styring et al., 2014a). The  $\delta^{15}\text{N}_{\text{Phe}}$  discrepancy between pigs and herbivores could also represent the separated spaces of herding, probably keeping pigs closer to the settlement and grazing herbivores further away, possibly more in upland as suggested for the Early Neolithic context in Germany at Vaihingen (Bentley and Knipper, 2005). Regardless, whatever and wherever the pigs are fed, the AA result indicates a more herbivorous TP than previously considered on the basis of N bulk collagen and confirms a different plant-based diet than herbivores. Analysis of more pigs would provide insight into whether this individual is representative of its community or has particularly low TP compared to others. If pigs are effectively more herbivorous than previously assumed (possibly feeding on manured cereals), the particularly high TP of humans is quite unexpected. A comparable case was found for the first Anatomically Modern Humans in Crimea: their high  $\delta^{15}\text{N}_{\text{bulk}}$  values supposed a high TP (Drucker et al., 2017). However, CSIA-AA has nuanced this result showing that mammoths' TP was actually close to 2 as herbivorous despite their high  $\delta^{15}\text{N}_{\text{bulk}}$  values (like GLN pigs) and that the humans' TP were closer to 2.5, suggesting a rather omnivorous

diet. In our case however, human TP is still high, most likely indicating a very carnivorous diet, or the introduction in diet of another resource of higher TP, like fish. This brings us to the second food item considered.

Regarding fish consumption, it is unlikely to be significant for these humans although it is not possible to exclude it, namely for some of them (as in particular GLN 294), occasionally. First, substantial consumption of freshwater fish such as pikes, carps or eels (supposed to be fished in this region) would result *a priori* in higher TP. Indeed, fish consumers are encountered regionally in the Mesolithic population of Noyen-sur-Seine (Paris basin) and present a TP higher than 3.5 (Naito et al., 2013a). Further East in Turkey, some Neolithic hunter-gatherers supposed to have eaten freshwater fish also recorded a TP higher than 3 (Itahashi et al., 2017). However, it is difficult to estimate the proportion of fish in the case of a mixed terrestrial and aquatic diet (Ohkouchi et al., 2017). A new method has been developed to disentangle fish consumption based on the difference between  $\delta^{15}\text{N}$  of phenylalanine and methionine ( $\delta^{15}\text{N}_{\text{Met}}$ ; Ishikawa et al., 2018). However, our analyses were conducted before this new development and we did not measure methionine. Another argument for no (or low) fish consumption concerns the  $\delta^{15}\text{N}_{\text{Phe}}$  values. The local range of values for freshwater fish is not known, but it is likely to be different from those of terrestrial animals, probably lower, as it is the case in other contexts. For example,  $\delta^{15}\text{N}_{\text{Phe}}$  values are systematically and significantly lower for freshwater fish than for terrestrial animals at the nearby Mesolithic site of Noyen-sur-Seine (Naito et al., 2013a), in Neolithic Turkey (Itahashi et al., 2019) or in Bronze-Iron age populations of Kazakhstan (Itahashi et al., 2020). Moreover, when humans from these sites consumed fish, they consistently had lower  $\delta^{15}\text{N}_{\text{Phe}}$  values than terrestrial animals. In our study, the human  $\delta^{15}\text{N}_{\text{Phe}}$  values are similar to those of terrestrial domestic animals, and they are contrarily different from those of wild species (assuming that two samples are representative of a whole). We can guess that freshwater resources could be distinct from domestic fauna in their  $\delta^{15}\text{N}_{\text{Phe}}$  values. In this case, the significant consumption of freshwater resources is unlikely.

Other omnivorous or carnivorous wild animals could have been consumed to complement the staple diet of humans, as for example badger, fox, birds or eggs, and the considerations are probably the same as for fish. Regardless, zooarchaeological data does not support a substantial intake of these resources and if they were consumed, that could be in a very occasional way which probably would not register significantly into the bones.

On another note, while animals'  $\delta^{15}\text{N}_{\text{Glu}}$  and  $\delta^{15}\text{N}_{\text{Phe}}$  values are well correlated ( $r \approx 0.9$ ), the humans'  $\delta^{15}\text{N}_{\text{Glu}}$  and  $\delta^{15}\text{N}_{\text{Phe}}$  values are rather negatively correlated (although the correlation coefficient  $r$  is very low, around -0.4) as are the  $\delta^{15}\text{N}_{\text{bulk}}$  and  $\delta^{34}\text{S}_{\text{bulk}}$  values of all the humans from GLN ( $r \approx -0.3$ ). This could indicate a relative reliance on freshwater fish supplies for individuals that have the highest TP and both the lowest  $\delta^{15}\text{N}_{\text{Phe}}$  and  $\delta^{34}\text{S}_{\text{bulk}}$  (assuming that fish should have lower  $\delta^{15}\text{N}_{\text{Phe}}$  and  $\delta^{34}\text{S}_{\text{bulk}}$  than terrestrial animals, as in other contexts). However, this could also indicate the relative reliance on plants vs. animal products, where individuals with the highest  $\delta^{15}\text{N}_{\text{Phe}}$  and  $\delta^{34}\text{S}_{\text{bulk}}$  and the lowest TP could have consumed more cereals. In this regard,  $\delta^{15}\text{N}_{\text{bulk}}$  and  $\delta^{15}\text{N}_{\text{Glu}}$  are well correlated in both humans and animals ( $r \approx 0.9$ ), indicating the trophic effect, and  $\delta^{34}\text{S}_{\text{bulk}}$  and  $\delta^{15}\text{N}_{\text{Phe}}$  are also well correlated in humans ( $r \approx 0.7$ ), rather indicating separated environments or different plants consumed. Although there is no sufficient data for both  $\delta^{34}\text{S}_{\text{bulk}}$  and  $\delta^{15}\text{N}_{\text{Phe}}$  in animals to track any direct correlation, it is noticeable that, considering the whole groups, domestic herbivores have both lower  $\delta^{34}\text{S}_{\text{bulk}}$  and  $\delta^{15}\text{N}_{\text{Phe}}$  values than pigs. Possibly, pigs were relying on human manured cereals waste. According to this hypothesis, cereals would have higher  $\delta^{34}\text{S}_{\text{bulk}}$  and  $\delta^{15}\text{N}_{\text{Phe}}$  values than herbivores' forage.

It is indisputable that plants constitute another end-member resource for caloric intake although their isotopic signature and their relative proportion in the diet are unknown. While Styring and colleagues

(2015) estimate that the plants'  $\delta^{15}\text{N}_{\text{Phe}}$  values of Vaihingen is similar to that of herbivores, in our context it is possible that this value approaches that of pigs, if pigs were consuming manured grain, and could be a better indication of the  $\delta^{15}\text{N}_{\text{Phe}}$  value of plant material consumed by humans (Fig. 4). Actually, both  $\delta^{15}\text{N}_{\text{Phe}}$  and  $\delta^{15}\text{N}_{\text{Glu}}$  values and consequently the  $\beta$  factor are quite variable in plants (see Appendix Fig. A1.f) and their local baseline clearly influences the subsequent interpretation. For instance, as Styring and colleagues (2015) suggest for Vaihingen, cereals' TP could be quite a bit higher than 1 thus leading to an increase in the resulting TP of humans. In this case, the humans' diet could be less carnivorous than assumed.

Thus, while CSIA-AA can be very useful to distinguish terrestrial from freshwater resources in some cases (low TP<2.5 vs. high TP>>3 + low  $\delta^{15}\text{N}_{\text{Phe}}$ ), it appears to be not so conclusive in the case of intermediate values with a mixed diet. Our results find an interesting example of comparison in Turkish Neolithic sites. On the one hand, Pre-Pottery Neolithic hunter-gatherers (from Hayankeyf Höyük) with high TP<sub>ter</sub> (>3) and low  $\delta^{15}\text{N}_{\text{Phe}}$  values have consumed freshwater fish: the amino acid  $\delta^{15}\text{N}$  analysis brings a quite clear picture (Itahashi et al., 2017). On the other hand, the Pottery Neolithic farmers from the same Upper Tigris Valley (at Hakemi Use) have shown lower TP<sub>ter</sub> ( $2.5 \pm 0.1$ ) and higher  $\delta^{15}\text{N}_{\text{Phe}}$  values, similar to those of terrestrial animals, suggesting a limited intake of aquatic protein (Itahashi et al., 2019). By comparison, our results would fall between these two observed patterns. Another case similar to the humans of GLN was observed for Neanderthals from Les Cottés and La Grotte du Renne (France; Jaouen et al., 2019). In this example, the combined analysis of the  $\delta^{13}\text{C}_{\text{Phe}}$  and  $\delta^{13}\text{C}_{\text{Val}}$  has achieved disentangling the question. These Neanderthals probably did not consume freshwater fish and the high TP (2.9) is interpreted as merely corresponding to a pure carnivorous diet.

To test our hypotheses and with the goal of broadening the discussion, we tried to model the relative contribution of different food sources with FRUITS software (Fernandes et al., 2014). All the input parameters, results and discussion are provided in Appendix B1 (text B1, Table B1 and Fig. B1). Due to several uncertainties inherent to the context and method (variable offsets, TDF and  $\beta$  value, unknown local food source values and food sources themselves, variable concentrations), the models are not expected to represent the true proportion of foodstuffs in the diet but they provide an interesting insights for further discussion of the interpretations. Moreover, they offer an opportunity to consider together different proxies: here we consider alternatively CNS isotope ratios, N isotope ratio in Glu and Phe and, for the first time to our knowledge, all these proxies together. In each case the models are based on 3 or 4 sources: plants, domestic herbivores, pigs and freshwater fish. The results indicate for the GluPhe model a lower plant intake, a higher meat intake (particularly of pig) and a lower freshwater fish intake than through the CNS model (Appendix Fig. B1). The model integrating all proxies globally shows intermediate results confirming our previous interpretations and enhances the inter-individual differences. Furthermore, it emphasizes the minimal contribution of freshwater fish to the diet of all individuals when considering the 4 end-members previously described. A more in-depth discussion is available in Appendix B1.

#### 4. CONCLUSIONS

To conclude, the CSIA-AA results, in addition to bulk collagen isotopic data, suggest a more carnivorous diet than previously supposed for most humans at Gurgy (GLN). These farmers certainly had a mainly terrestrial based diet, with domestic animals as the primary protein intake and plants (possibly manured cereals) as a food supplement. Pigs were likely more herbivorous than assumed, possibly feeding on manured cereals from human food waste. By the way, cereals could present a

higher TP than assumed and the variability of  $\beta$  factor and TDF needs to be further defined. Nevertheless, freshwater fish were apparently not a significant contribution to the human diet although their occasional consumption is not definitely excluded for some individuals. On the other hand, the results confirm low TP for a woman previously highlighted as outlier and support a rather omnivorous diet for the one human analyzed at Pontcharaud (NFN). Finally, the direct comparison with local freshwater fish and more pig samples could confirm these results.

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## **CONFLICT OF INTEREST**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## **DATA AVAILABILITY STATEMENT**

The authors declare that the data supporting the findings of this study are all available within the article and its supporting information (SI) files.

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## TABLE AND FIGURE CAPTIONS

**Fig. 1:** Location map of the sites analyzed in this study.

**Fig. 2:** Distribution of C, N and S isotopic values of the samples analyzed in this study relative to the global variability of all the humans and animals from these sites and relative to plant and freshwater fish values from literature as an estimate (Estim.) for comparison. Plants (cereals and pulses) C and N values are from four Neolithic sites in Germany (Styring et al., 2017). S values are estimated from those of herbivores. Freshwater fishes are from various earlier or contemporaneous archaeological sites from neighboring regions (see Appendix Table A1-7 and Fig. A1; Bösl et al., 2006; Drucker and Bocherens, 2004; Drucker et al., 2016; Drucker et al., 2018; Dürrwächter et al., 2006; Meadows et al., 2018). dom.=domestic, herbiv.=herbivores

**Fig. 3:** Schematic illustration of the relationship between trophic position (TP) of organisms and their nitrogen isotopic values of Glu and Phe in C3-plant-based (A) and aquatic (B) ecosystems (modified after Chikaraishi et al., 2011). The  $\Delta^{15}\text{N}_{\text{Glu-Phe}}$  value in primary producers ( $\beta$  factor) varies among C3 plants (-8.4‰) and aquatic plants such as algae or cyanobacteria (+3.4‰; Chikaraishi et al., 2007; Chikaraishi et al., 2010a).

**Fig. 4:** Distribution of  $\delta^{15}\text{N}_{\text{Glu}}$  vs.  $\delta^{15}\text{N}_{\text{Phe}}$  values of human and animal samples analyzed in this study relative to human and freshwater fish values from the French sites Noyen-sur-Seine and Pont d'Ambon(Naito et al., 2013a) and relative to estimated plant values from the German Neolithic site of Vaihingen for  $\delta^{15}\text{N}_{\text{Glu}}$ (Styring et al., 2015) and from the mean of human and domestic animal  $\delta^{15}\text{N}_{\text{Phe}}$  values of our samples minus the theoretical trophic enrichment of 0.4‰ for  $\delta^{15}\text{N}_{\text{Phe}}$ .

**Table 1:** Archaeological and biological data of the sample selected for the CSIA-AA and complementary analytical information. \*sex determined by genetics (Rivollat, pers. comm.) 1: Chambon et al., 2013, 2: Rivollat et al., 2015, 3: Rottier et al., 2005, 4: Rottier, 2007, 5: Coutelas et al., 2015, 6: Loison, 1998, 7: Rey et al., 2019, 8: Rey et al., 2017, 9: Goude et al., 2019, 10: Goude et al., 2013, 11: Goude et al., 2021

**Table 2:** Carbon, nitrogen and sulphur isotopic values of bulk collagen, nitrogen isotopic values of single amino acids and corresponding trophic position in terrestrial C3-plant based ecosystem (Ter) and aquatic ecostem (Aqua). 1: Rey et al. 2019, 2: Rey et al. 2017, 3: Goude et al. 2019, 4: Goude et al. 2013, 5: Goude et al. 2021

## APPENDIX CAPTIONS

**Fig. A1:** Isotopic variability of terrestrial plants and freshwater fish values available in literature from close contexts: **a)** Distribution of C and N bulk isotopic values of cereal grains and pulse seeds from four Neolithic sites in Germany (Styring et al., 2017) and **b)** location map of the corresponding sites. **c)** Distribution of C and N bulk isotopic values and **e)** N and S isotopic values of freshwater fish samples from various «neighbouring» sites (Bösl et al., 2006; Drucker and Bocherens, 2004; Drucker et al., 2016; Drucker et al., 2018; Dürrwächter et al., 2006; Meadows et al., 2018), Turkish site (Itahashi et al., 2019) and freshwater fish and mussel modern samples (Fernandes et al., 2015) ; **d)** location map of the corresponding sites.**f)** Distribution of N isotopic values of single amino acids of different modern plant samples from various sites (Bol et al., 2002; Itahashi et al., 2019; Kendall et al., 2019; Paolini et al., 2015; Steffan et al., 2013; Styring et al., 2015; Styring et al., 2014; Takizawa et al., 2017; Yoneyama and Tanaka, 1999) and **g)** of freshwater fish samples from the Epipaleolithic site of Pont d'Ambon(Naito et al., 2013) and the Neolithic Turkish site of HasankeyfHöyük(Itahashi et al., 2019).

**Fig. A2:** Distribution of C, N and S isotopic values of the samples analyzed in this study by CSIA-AA relative to all the samples previously analyzed for the corresponding sites (Goude et al., 2019; Goude et al., 2013; Rey et al., 2017; Rey et al., 2019). **a.**  $\delta^{13}\text{C}$  vs  $\delta^{15}\text{N}$  values from the Yonne region, **b.**  $\delta^{13}\text{C}$  vs  $\delta^{15}\text{N}$  values from Le Vigneau, **c.**  $\delta^{13}\text{C}$  vs  $\delta^{15}\text{N}$  values from Pontcharaud, **d.**  $\delta^{34}\text{S}$  vs  $\delta^{15}\text{N}$  values from the Yonne region and Pontcharaud, **e.**  $\delta^{34}\text{S}$  vs  $\delta^{15}\text{N}$  values from Le Vigneau.

**Table A1-7:** Summary of all the archaeological and isotopic data used as baseline for the models. 8 sheets: 1 baseline for the Yonne region, 1 baseline for Pontcharaud, 1 baseline for Le Vigneau (LV), 1 baseline for freshwater resources (FW), 1 baseline for riparians, 1 baseline for plants, 1 summary of average plant values, 1 baseline for concentrations of food sources from USDA(Haytowitz et al., 2019).

**Appendix B1:** [text] FRUITS models, baseline reference and parameters, results and discussion

**Table B1:** Summary of input parameters for FRUITS models.

**Fig. B1:** Model estimates of the relative contribution in the diet of GLN and NFN individuals of different food sources (in mass) considered under specific conditions determined by the input parameters given in SI\_models. Models are based on C, N and S isotope ratios of bulk collagen (A and E); N isotope ratios of Glu and Phe for 3 different sources (B-C and F); all proxies combined C, N and S isotope ratios of bulk collagen and N isotope ratios of Glu and Phe (D and G), for individuals from GLN (A-D) and NFN (E-G). Boxes represent a 68% credible interval (corresponding to the 16th and 84th percentiles) while the whiskers represent a 95% credible interval (corresponding to the 2.5th and 97.5th percentiles). The horizontal continuous line represents the estimated mean while the horizontal discontinuous line represents the estimated median (50th percentile).

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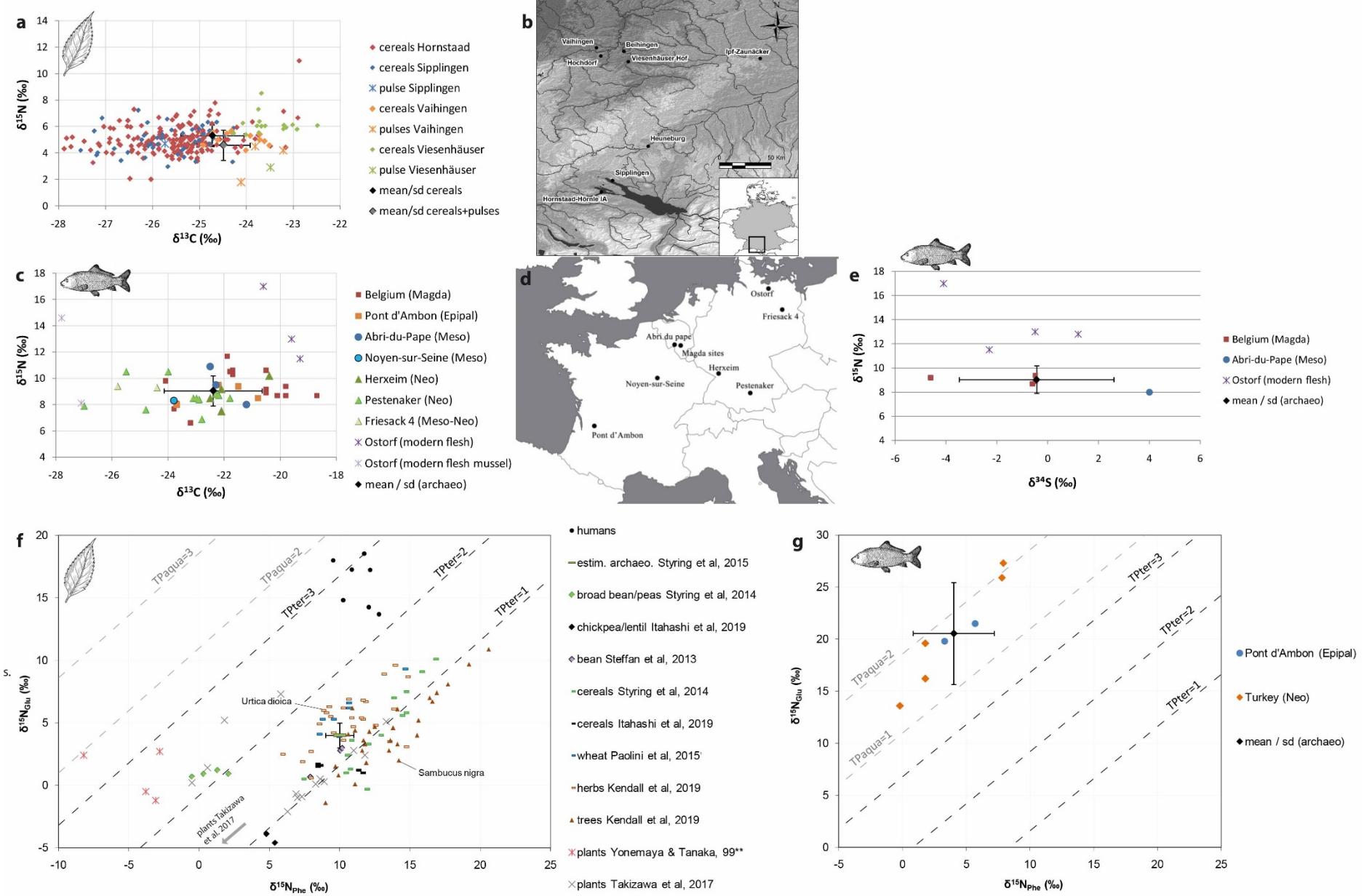
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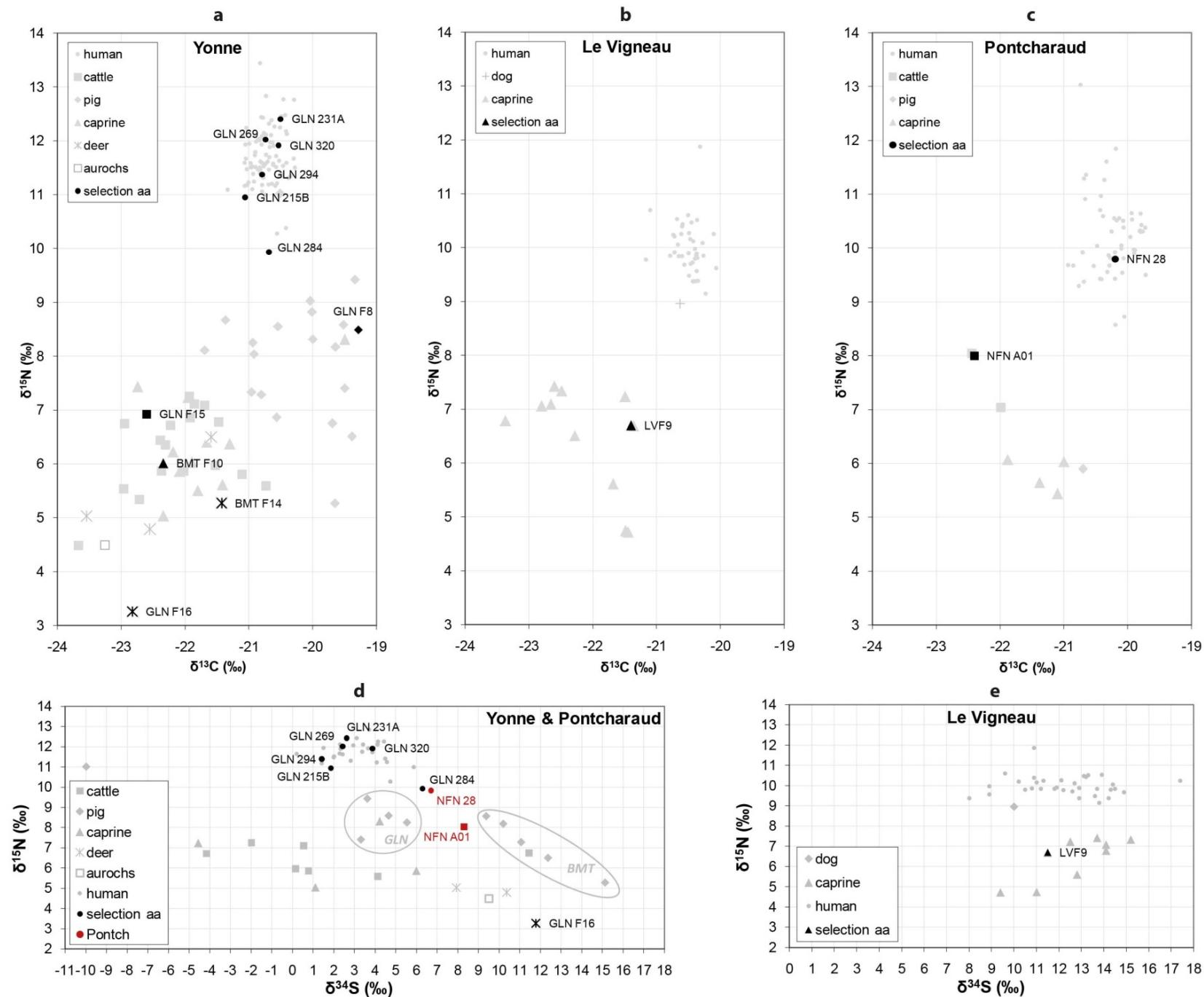
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**Figure S1**



**Figure S1:** Isotopic variability of terrestrial plants and freshwater fish values available in literature from close contexts: **a)** Distribution of C and N bulk isotopic values of cereal grains and pulse seeds from four Neolithic sites in Germany (Styring et al., 2017) and **b)** location map of the corresponding sites. **c)** Distribution of C and N bulk isotopic values and **e)** N and S isotopic values of freshwater fish samples from various «neighbouring» sites (Bösl et al., 2006; Drucker and Bocherens, 2004; Drucker et al., 2016; Drucker et al., 2018; Dürrwächter et al., 2006; Meadows et al., 2018), Turkish site (Itahashi et al., 2019) and freshwater fish and mussel modern samples (Fernandes et al., 2015) ; **d)** location map of the corresponding sites.**f)** Distribution of N isotopic values of single amino acids of different modern plant samples from various sites (Bol et al., 2002; Itahashi et al., 2019; Kendall et al., 2019; Paolini et al., 2015; Steffan et al., 2013; Styring et al., 2015; Styring et al., 2014; Takizawa et al., 2017; Yoneyama and Tanaka, 1999) and **g)** of freshwater fish samples from the Epipaleolithic site of Pont d’Ambon (Naito et al., 2013) and the Neolithic Turkish site of Hasankeyf Höyük (Itahashi et al., 2019).

**Figure S2**



**Figure S2:** Distribution of C, N and S isotopic values of the samples analyzed in this study by CSIA-AA relative to all the samples previously analyzed for the corresponding sites (Goude et al., 2019; Goude et al., 2013; Rey et al., 2017; Rey et al., 2019). **a.**  $\delta^{13}\text{C}$  vs  $\delta^{15}\text{N}$  values from the Yonne region, **b.**  $\delta^{13}\text{C}$  vs  $\delta^{15}\text{N}$  values from Le Vigneau, **c.**  $\delta^{13}\text{C}$  vs  $\delta^{15}\text{N}$  values from Pontcharaud, **d.**  $\delta^{34}\text{S}$  vs  $\delta^{15}\text{N}$  values from the Yonne region and Pontcharaud, **e.**  $\delta^{34}\text{S}$  vs  $\delta^{15}\text{N}$  values from Le Vigneau

Table S1

ID	site	species (*dom ?)	model source	archaeo ID	AGE at death	age group	site localisation	analyzed bone	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\delta^{34}\text{S}$ (‰)	reference	$\delta^{15}\text{N}_{\text{Glu}}$ (‰)	$\delta^{15}\text{N}_{\text{Phe}}$ (‰)
GLN_F0	Gurgy "Les Noisats"	cattle*	herbiv dom			?		mandib	-23.0	6.7	11.4	Rey et al. 2017;		
GLN_F1	Gurgy "Les Noisats"	cattle	herbiv dom	near st 234 (burial)		-		long bone	-21.7	7.1		Rey et al. 2019		
GLN_F3	Gurgy "Les Noisats"	cattle	herbiv dom	st 253-5 (burial)		-		mandib	-21.5	6.8		Rey et al. 2019		
GLN_F4	Gurgy "Les Noisats"	cattle	herbiv dom	st 275-1 (burial)	adult	adult		mandib	-21.9	7.3	-2.0	Rey et al. 2019		
GLN_F11	Gurgy "Les Noisats"	cattle	herbiv dom	st 38-soil 2	adult > 24-30 months	adult		MT	-21.9	6.9		Rey et al. 2019		
GLN_F13	Gurgy "Les Noisats"	caprine	herbiv dom	st 60-26	adult	adult		long bone	-19.5	8.3	4.2	Rey et al. 2019		
GLN_F14	Gurgy "Les Noisats"	cattle	herbiv dom	st 60-34	adult	adult		coxal	-22.4	5.9	0.8	Rey et al. 2019		
GLN_F15	Gurgy "Les Noisats"	cattle	herbiv dom	st 60-35	months but probably >12months)	juvenile		long bone	-22.6	6.9		Rey et al. 2019	10.3	10.8
BMT_F2	Beaumont "Le Crot aux Moines"	cattle	herbiv dom	Merlange C3.4.IX		-	area B - pit A	talus	-22.2	6.7	-4.2	Rey et al. 2019		
BMT_F6	Beaumont "Le Crot aux Moines"	caprine	herbiv dom	Merlange C2.21	juvenile < 50-55 months	juvenile	area B - pit A	long bone	-22.0	7.2	-4.5	Rey et al. 2019		
BMT_F8	Beaumont "Le Crot aux Moines"	cattle	herbiv dom	Merlange C6.4.XXXVIII		-	area B - pit A	talus	-21.1	5.8		Rey et al. 2019		
BMT_F9	Beaumont "Le Crot aux Moines"	cattle	herbiv dom	Merlange F4.30.XL		-	area B - pit B	talus	-22.0	5.9		Rey et al. 2019		
BMT_F10	Beaumont "Le Crot aux Moines"	caprine	herbiv dom	Merlange A2.5	adult	adult	area B - pit A	long bone	-22.3	6.0		Rey et al. 2019	8.5	9.2
BMT_F12	Beaumont "Le Crot aux Moines"	cattle	herbiv dom	Merlange E4.4.CLV		-	area B - pit B	talus	-20.7	5.6	4.1	Rey et al. 2019		
BMT_F15	Beaumont "Le Crot aux Moines"	caprine	herbiv dom	G6.2.CLXXXXIII	adult	adult	area B - pit B	long bone	-21.7	6.4		Rey et al. 2019		
BMT_F16	Beaumont "Le Crot aux Moines"	caprine	herbiv dom	Merlange F4.2.LXXXV		-	area B - pit B	skull	-22.3	5.0	1.1	Rey et al. 2019		
BMT_F17	Beaumont "Le Crot aux Moines"	cattle	herbiv dom	layer IV - M10.1.CLIX		-	layer IV	MT	-22.4	6.4		Rey et al. 2019		
BMT_F18	Beaumont "Le Crot aux Moines"	caprine	herbiv dom	layer IV - - - capr1	young adult	adult	layer IV	mandib	-21.8	5.5		Rey et al. 2019		
BMT_F19	Beaumont "Le Crot aux Moines"	caprine	herbiv dom	layer IV - O9.4.373 - capr2	between 12-40 months	juvenile	layer IV	long bone	-21.3	6.4		Rey et al. 2019		
BMT_F21	Beaumont "Le Crot aux Moines"	cattle	herbiv dom	layer 5 Ederlé - bos 1	young < 24-30 months	juvenile	Ederlé	MT	-23.0	5.5		Rey et al. 2019		
BMT_F22	Beaumont "Le Crot aux Moines"	cattle	herbiv dom	layer 5 Ederlé - bos 2	adult	adult	Ederlé	MT	-22.7	5.3		Rey et al. 2019		
BMT_F23	Beaumont "Le Crot aux Moines"	cattle	herbiv dom	layer 5 Ederlé - bos 3	adult	adult	Ederlé	MT	-23.7	4.5		Rey et al. 2019		
BMT_F24	Beaumont "Le Crot aux Moines"	cattle	herbiv dom	layer 5 Ederlé - bos 4			Ederlé	mandib	-21.9	7.1	0.5	Rey et al. 2019		
BMT_F26	Beaumont "Le Crot aux Moines"	caprine	herbiv dom	layer 5 Ederlé - capr 1	adult	adult	Ederlé	mandib	-21.4	5.6		Rey et al. 2019		
BMT_F27	Beaumont "Le Crot aux Moines"	caprine	herbiv dom	layer 5 Ederlé - capr 2	young adult	adult	Ederlé	mandib	-22.1	5.9	6.0	Rey et al. 2019		
BMT_F31	Beaumont "Le Crot aux Moines"	cattle	herbiv dom	circular house - -	adult	adult	house	talus	-22.3	6.4		Rey et al. 2019		
BMT_F32	Beaumont "Le Crot aux Moines"	caprine	herbiv dom	circular house - -	<1 year	juvenile	house	mandib	-21.9	6.0		Rey et al. 2019		
BMT_F46	Beaumont "Le Crot aux Moines"	caprine	herbiv dom	layer 5 Ederlé - capr			Ederlé	skull	-22.2	6.2		Rey et al. 2019		
NZ_F1	Gurgy "Le Nouzeau"	cattle	herbiv dom	88.Q.5		-		long bone	-21.5	6.0	0.1	Rey et al. 2019		
NZ_F2	Gurgy "Le Nouzeau"	caprine	herbiv dom	157.8		-		MT	-22.7	7.4		Rey et al. 2019		
GLN_F16	Gurgy "Les Noisats"	roe deer	herbiv wild	st 60-36	adult	adult		MT	-22.8	3.3	11.8	Rey et al. 2019	5.3	6.0
BMT_F11	Beaumont "Le Crot aux Moines"	red deer	herbiv wild	Merlange G4.4.XXXIV		-	area B - pit B	talus	-22.6	4.8	10.4	Rey et al. 2019		
BMT_F14	Beaumont "Le Crot aux Moines"	red deer	herbiv wild	Merlange D4.9.X2		-	area B - pit A	talus	-21.4	5.3		Rey et al. 2019	8.8	9.8
BMT_F25	Beaumont "Le Crot aux Moines"	red deer	herbiv wild	layer 5 Ederlé -		-	Ederlé	long bone	-21.6	6.5		Rey et al. 2019		
NZ_F4	Gurgy "Le Nouzeau"	red deer	herbiv wild	276.15		-		long bone	-23.5	5.0	7.9	Rey et al. 2019		
NZ_F5	Gurgy "Le Nouzeau"	aurochs	herbiv wild	322.29		-		horn core	-23.3	4.5	9.5	Rey et al. 2019		
BMT_F1	Beaumont "Le Crot aux Moines"	pig	pig BMT	Merlange I4.2.CCXXX		-	area B - pit B	long bone	-20.0	8.3		Rey et al. 2019		
BMT_F3	Beaumont "Le Crot aux Moines"	pig	pig BMT	Merlange B3.4.XV	adult	adult	area B - pit A	long bone	-19.6	8.2	10.2	Rey et al. 2019		

ID	site	species (*dom ?)	model source	archaeo ID	AGE at death	age group	site localisation	analyzed bone	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{34}\text{S}$	reference	$\delta^{15}\text{N}_{\text{Glu}}$	$\delta^{15}\text{N}_{\text{Phe}}$
									(‰)	(‰)	(‰)		(‰)	(‰)
BMT_F4	Beaumont "Le Crot aux Moines" pig	pig BMT	Merlange H6.2.CCXIX	very young		juvenile	area B - pit B	long bone	-20.0	8.8		Rey et al. 2019		
BMT_F5	Beaumont "Le Crot aux Moines" pig	pig BMT	Merlange I7.4.CCXXXIII				area B - pit B	long bone	-20.5	11.0	-10.0	Rey et al. 2019		
BMT_F7	Beaumont "Le Crot aux Moines" pig	pig BMT	Merlange M8.298	adult		adult	area B - pit B	long bone	-20.0	9.0		Rey et al. 2019		
BMT_F13	Beaumont "Le Crot aux Moines" pig	pig BMT	M5.CCLXXXVII				area B - pit B	long bone	-20.8	7.3	11.1	Rey et al. 2019		
BMT_F20	Beaumont "Le Crot aux Moines" pig	pig BMT	layer IV - DNW				layer IV	long bone	-21.0	7.3		Rey et al. 2019		
BMT_F29	Beaumont "Le Crot aux Moines" pig	pig BMT	layer 5 Ederlé - sus 1	adult		adult	Ederlé	MT	-20.5	8.6	9.4	Rey et al. 2019		
BMT_F30	Beaumont "Le Crot aux Moines" pig	pig BMT	layer 5 Ederlé - sus 2	adult		adult	Ederlé	long bone	-20.9	8.0		Rey et al. 2019		
BMT_F33	Beaumont "Le Crot aux Moines" pig	pig BMT	circular house - sus 1	young adult		adult	house	long bone	-19.7	5.3	15.1	Rey et al. 2019		
BMT_F34	Beaumont "Le Crot aux Moines" pig	pig BMT	circular house - sus 2				house	long bone	-19.4	6.5	12.4	Rey et al. 2019		
BMT_F41	Beaumont "Le Crot aux Moines" pig	pig BMT	Merlange E3.3.XLVIII	adult		adult	area B - pit A	skull	-21.7	8.1		Rey et al. 2019		
BMT_F42	Beaumont "Le Crot aux Moines" pig	pig BMT	A"02.1.CXXXVII	adult		adult	area B - pit A	mandib	-21.4	8.7		Rey et al. 2019		
BMT_F44	Beaumont "Le Crot aux Moines" pig	pig BMT	Merlange B3.7.XX	juvenile		juvenile	area B - pit A	skull	-20.6	6.9		Rey et al. 2019		
GLN_F6	Gurgy "Les Noisats"	pig	pig Gurgy	st 38-6	young (weaned ?)	juvenile		mandib	-20.9	8.2	5.5	Rey et al. 2019		
GLN_F7	Gurgy "Les Noisats"	pig	pig Gurgy	st 38-13		?		mandib	-19.5	8.6	4.6	Rey et al. 2019		
GLN_F8	Gurgy "Les Noisats"	pig	pig Gurgy	st 38-14		?		coxal	-19.3	8.5		Rey et al. 2019	12.9	12.6
GLN_F9	Gurgy "Les Noisats"	pig	pig Gurgy	st 38-19		?		mandib	-19.5	7.4	3.3	Rey et al. 2019		
GLN_F17	Gurgy "Les Noisats"	pig	pig Gurgy	st 60-42		-		long bone	-19.3	9.4	3.6	Rey et al. 2019		
NZ_F3	Gurgy "Le Nouzeau"	pig*	pig Gurgy	194.28		-		long bone	-19.7	6.8		Rey et al. 2019		

**Table S1: Archaeological and isotopic data of the animal samples considered for the Yonne baseline, from three Neolithic sites: GLN= Gurgy "Les Noisats", BMT = Beaumont "Le Crot aux Moines", NZ = Gurgy "Le Nouzeau".**

Rey L, Goude G, Rottier S (2017) Comportements alimentaires au Néolithique : nouveaux résultats dans le Bassin parisien à partir de l'étude isotopique ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ) de la nécropole de Gurgy « Les Noisats » (Yonne, Ve millénaire av. J.-C.). Bulletins et Mémoires de la Société d'Anthropologie de Paris 29:54-69. doi:10.1007/s13219-016-0170-9

Rey, L., D. C. Salazar-García, P. h. Chambon, F. Santos, S. Rottier, and G. Goude, 2019, A multi-isotope analysis of Neolithic human groups in the Yonne valley, Northern France: insights into dietary patterns and social structure, Archaeological and Anthropological Sciences, 11(10), 5591–5616.

ID	site	species	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{34}\text{S}$	reference	$\delta^{15}\text{N}_{\text{Glu}}$	$\delta^{15}\text{N}_{\text{Phe}}$
			(‰)	(‰)	(‰)		(‰)	(‰)
NFNA01	Pontcharaud 2	cattle	-22.4	8.0	8.3	Goude et al. 2013;		
NFNA07	Pontcharaud 2	cattle	-22.0	7.0		Goude et al. 2021	10.3	11.1
NFNA02	Pontcharaud 2	caprine	-21.9	6.1		Goude et al. 2013		
NFNA03	Pontcharaud 2	caprine	-21.1	5.4		Goude et al. 2013		
NFNA08	Pontcharaud 2	caprine	-21.4	5.7		Goude et al. 2013		
NFNA05	Pontcharaud 2	caprine	-21.0	6.0		Goude et al. 2013		
NFNA04	Pontcharaud 2	pig	-20.7	5.9		Goude et al. 2013		

**Table S2: Archaeological and isotopic data of the animal samples from the site of Pontcharaud2 (NFN)**

Goude, G., A. Schmitt, E. Herrscher, G. Loison, S. Cabut, and G. André, 2013, Pratiques alimentaires au Néolithique moyen : nouvelles données sur le site de Pontcharaud 2 (Puy-de-Dôme, Auvergne, France), Bulletin de la Société préhistorique française, 110(2), 299-317.

Goude, G., M. Rivollat, A. Schmitt, D. Salazar-García, S. Villotte, A. Lambert, A. Sanz, P. Brige, M.-H. Pemonge, W. Haak, and M.-F. Deguilloux, 2021, Identifying biological and behavioural diversity among European Neolithic farmers: 9th International Symposium on Biomolecular Archaeology (1er-4 juin, Toulouse, France). Poster presentation.

ID	site	species	age	age class	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{34}\text{S}$	reference	$\delta^{15}\text{N}_{\text{Glu}}$	$\delta^{15}\text{N}_{\text{Phe}}$
					(‰)	(‰)	(‰)		(‰)	(‰)
LV F01	Le Vigneau 2	Lamb	3 months	juvenile	-23.4	6.8	14.1	Goude et al. 2019		
LV F02	Le Vigneau 2	Lamb	New born	new born	-21.5	7.2	12.5	Goude et al. 2019		
LV F03	Le Vigneau 2	Lamb	3 months	juvenile	-21.4	4.7	9.4	Goude et al. 2019		
LV F04	Le Vigneau 2	Lamb	3 months	juvenile	-21.5	4.7	11.0	Goude et al. 2019		
LV F05	Le Vigneau 2	Lamb	New born	new born	-22.8	7.1		Goude et al. 2019		
LV F06	Le Vigneau 2	Lamb	9 months	juvenile	-21.7	5.6	12.8	Goude et al. 2019		
LV F07	Le Vigneau 2	Lamb	9 months	juvenile	-22.3	6.5		Goude et al. 2019		
LV F08	Le Vigneau 2	Lamb	New born	new born	-22.7	7.1	14.1	Goude et al. 2019		
LV F09	Le Vigneau 2	Sheep	18 months	juvenile	-21.4	6.7	11.5	Goude et al. 2019	9.5	11.9
LV F10	Le Vigneau 2	Dog	1-2 yrs	juvenile	-20.6	9.0	10.0	Goude et al. 2019		
LV F11	Le Vigneau 2	Lamb	9 months	juvenile	-22.6	7.4	13.7	Goude et al. 2019		
LV F12	Le Vigneau 2	Lamb	New born	new born	-22.5	7.3	15.2	Goude et al. 2019		

**Table S3: Archaeological and isotopic data of the animal samples from the site of Le Vigneau 2 (LV)**

Goude, G., D. C. Salazar-García, R. C. Power, J. Terrom, M. Rivollat, M.-F. Deguilloux, M.-H. Pemonge, M. Le Bailly, G. Andre, A. Coutelas, and A. Hauzeur, 2019, A Multidisciplinary Approach to Neolithic Life Reconstruction, Journal of Archaeological Method and Theory, 26, 537–560.

Table S4

site	species	lat species	site localisation	period	fraction	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{34}\text{S}$	reference	$\delta^{15}\text{N}_{\text{Glu}}$	$\delta^{15}\text{N}_{\text{Phe}}$	TPter	TPaqua	reference	
						(‰)	(‰)	(‰)		(‰)	(‰)				
Abri-du-Pape	pike	<i>Esox lucius</i>	Belgium	Meso	collagen	-22.5	10.9		Drucker et al, 2018						
Abri-du-Pape	cyprinidae	Cyprinidae	Belgium	Meso	collagen	-21.2	8		4 Drucker et al, 2018						
Abri-du-Pape	cyprinidae	Cyprinidae	Belgium	Meso	collagen	-22.3	9.5		Drucker et al, 2018						
Trou du Sureau	burbot	<i>Lota lota</i>	Belgium	Magda ?	collagen	-23.2	6.6		Drucker et al, 2016						
Trou du Frontal	burbot	<i>Lota lota</i>	Belgium	Magda ?	collagen	-21.8	10.5		Drucker et al, 2016						
Bois Laiterie	burbot	<i>Lota lota</i>	Belgium	Magda ?	collagen	-24.1	9.8		Drucker et al, 2016						
Bois Laiterie	burbot	<i>Lota lota</i>	Belgium	Magda ?	collagen	-23.8	7.7		Drucker et al, 2016						
Trou de Chaleux	brown trout	<i>Salmo trutta fario</i>	Belgium	Magda ?	collagen	-18.7	8.7		Drucker et al, 2016						
Bois Laiterie	brown trout	<i>Salmo trutta fario</i>	Belgium	Magda ?	collagen	-20.5	9.2		-4.6 Drucker et al, 2016; 2018						
Bois Laiterie	brown trout	<i>Salmo trutta fario</i>	Belgium	Magda ?	collagen	-19.8	9.4		-0.5 Drucker et al, 2016; 2018						
Bois Laiterie	brown trout	<i>Salmo trutta fario</i>	Belgium	Magda ?	collagen	-20.5	8.9		-0.5 Drucker et al, 2016; 2018						
Bois Laiterie	brown trout	<i>Salmo trutta fario</i>	Belgium	Magda ?	collagen	-19.8	8.7		Drucker et al, 2016						
Trou du Frontal	pike	<i>Esox lucius</i>	Belgium	Magda ?	collagen	-20.5	10.6		Drucker et al, 2016						
Trou du Frontal	pike	<i>Esox lucius</i>	Belgium	Magda ?	collagen	-23.7	8.1		Drucker et al, 2016						
Trou de Chaleux	pike	<i>Esox lucius</i>	Belgium	Magda ?	collagen	-21.7	10.3		Drucker et al, 2016						
Trou de Chaleux	nase	<i>Chondrostoma nasus</i>	Belgium	Magda ?	collagen	-20.1	8.7		-0.6 Drucker et al, 2016; 2018						
Trou du Frontal	cyprinidae	Cyprinidae	Belgium	Magda ?	collagen	-21.9	11.7		Drucker et al, 2016						
Trou du Frontal	cyprinidae	Cyprinidae	Belgium	Magda ?	collagen	-21.7	10.6		Drucker et al, 2016						
Trou du Frontal	cyprinidae	Cyprinidae	Belgium	Magda ?	collagen	-21.8	10.5		Drucker et al, 2016						
Herxheim	pike	<i>Esox lucius</i>	(Germany)	Early Neo	collagen	-22.1	9.2		Dürrwächter et al, 2006						
Herxheim	pike	<i>Esox lucius</i>	(Germany)	Early Neo	collagen	-22.5	8.5		Dürrwächter et al, 2006						
Herxheim	carp	<i>Cyprinus sp.</i>	(Germany)	Early Neo	collagen	-20.4	10.2		Dürrwächter et al, 2006						
Herxheim	carp	<i>Cyprinus sp.</i>	(Germany)	Early Neo	collagen	-22.1	7.5		Dürrwächter et al, 2006						
Noyen-sur-Seine	"Haut des Nâchères"	eel	<i>Anguilla anguilla</i>	Seine-et-Marne (France)	Meso	collagen	-23.8	8.3		Drucker & Bocherens, 2004					
Pestenacker	barbel	<i>Barbus barbus</i>	Bavaria (Germany)	Late Neo	collagen	-23.1	8.5		Bösl et al, 2006						
Pestenacker	carp	<i>Cyprinus carpio</i>	Bavaria (Germany)	Late Neo	collagen	-27	7.9		Bösl et al, 2006						
Pestenacker	cyprinidae	Cyprinidae	Bavaria (Germany)	Late Neo	collagen	-24.8	7.6		Bösl et al, 2006						
Pestenacker	cyprinidae	Cyprinidae	Bavaria (Germany)	Late Neo	collagen	-21.8	8.5		Bösl et al, 2006						
Pestenacker	catfish	<i>Silurus glanis</i>	Bavaria (Germany)	Late Neo	collagen	-24	10.5		Bösl et al, 2006						
Pestenacker	chub	<i>Leuciscus cephalus</i>	Bavaria (Germany)	Late Neo	collagen	-23	8.5		Bösl et al, 2006						
Pestenacker	chub	<i>Leuciscus cephalus</i>	Bavaria (Germany)	Late Neo	collagen	-22.3	8.8		Bösl et al, 2006						
Pestenacker	chub	<i>Leuciscus cephalus</i>	Bavaria (Germany)	Late Neo	collagen	-22.9	8.4		Bösl et al, 2006						
Pestenacker	pike	<i>Esox lucius</i>	Bavaria (Germany)	Late Neo	collagen	-22.8	6.9		Bösl et al, 2006						
Pestenacker	pike	<i>Esox lucius</i>	Bavaria (Germany)	Late Neo	collagen	-22.2	8.7		Bösl et al, 2006						
Pestenacker	pike	<i>Esox lucius</i>	Bavaria (Germany)	Late Neo	collagen	-25.5	10.5		Bösl et al, 2006						
Pont d'Ambron	pike	<i>Esox lucius</i>	France	Epipal	collagen	-22.2	9.5		Drucker & Bocherens, 2004	21.5	5.7			Naito et al, 2013	
Pont d'Ambron	carp	Cyprinidae	France	Epipal	collagen	-21.5	9.4		Drucker & Bocherens, 2004	19.8	3.3			Naito et al, 2013	

site	species	lat species	site localisation	period	fraction	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\delta^{34}\text{S}$ (‰)	reference	$\delta^{15}\text{N}_{\text{Glu}}$ (‰)	$\delta^{15}\text{N}_{\text{Phe}}$ (‰)	TPter	TPqua	reference
Pont d'Ambon	eel	<i>Anguilla anguilla</i>	France	Epipal	collagen	-23.7	8		Drucker & Bocherens, 2004					
Pont d'Ambon	eel	<i>Anguilla anguilla</i>	France	Epipal	collagen	-20.8	8.5		Drucker & Bocherens, 2004					
Friesack 4	eel	<i>Anguilla anguilla</i>	Brandenburg (Germany)	Meso-Neo	collagen	-24.4	9.3		Meadows et al, 2018					
Friesack 4	perch	<i>Perca fluviatilis</i>	Brandenburg (Germany)	Meso-Neo	collagen	-25.8	9.4		Meadows et al, 2018					
Ostorf	Pike	<i>Esox lucius</i>	Lake Schwerin (Germ)	modern	flesh - bulk	-19.6	13	-0.5	Fernandes et al, 2015					
Ostorf	Bream	<i>Abramis brama</i> ?	Lake Schwerin (Germ)	modern	flesh - bulk	-19.3	11.5	-2.3	Fernandes et al, 2015					
Ostorf	Eel	<i>Anguilla anguilla</i>	Lake Schwerin (Germ)	modern	flesh - bulk	-15	12.8	1.2	Fernandes et al, 2015					
Ostorf	Eel	<i>Anguilla anguilla</i>	Lake Schwerin (Germ)	modern	flesh - bulk	-20.6	17	-4.1	Fernandes et al, 2015					
Ostorf	Zebra mussel	<i>Dreissena polymorpha</i>	Lake Schwerin (Germ)	modern	flesh - bulk	-27.1	8.1	-	Fernandes et al, 2015					
Ostorf	Zebra mussel	<i>Dreissena polymorpha</i>	Lake Ostorf (Germ)	modern	flesh - bulk	-27.8	14.6	-	Fernandes et al, 2015					
Hasankeyf Höyük	Cyprinidae	Cyprinidae	Turkey	Neolithic		-24.1	8.8		Itahashi et al. 2019	13.6	-0.2	3.9	2.4	Itahashi et al. 2019
Hasankeyf Höyük	Cyprinidae	Cyprinidae	Turkey	Neolithic		-25.2	9		Itahashi et al. 2019	19.6	1.8	4.4	2.9	Itahashi et al. 2019
Hasankeyf Höyük	Cyprinidae	Cyprinidae	Turkey	Neolithic						25.9	7.8	4.5	2.9	Itahashi et al. 2019
Hasankeyf Höyük	Cyprinidae	Cyprinidae	Turkey	Neolithic						27.3	7.9	4.7	3.1	Itahashi et al. 2019
Hasankeyf Höyük	Barbus sp.	Barbus sp.	Turkey	Neolithic						16.2	1.8	4	2.5	Itahashi et al. 2019

**Table S4: Archaeological and isotopic data of the freshwater fish samples considered as baseline for the FRUITS models, from various neighboring sites of close context and from modern samples.**

Drucker, D. G., F. Valentin, C. Thevenet, D. Mordant, R. Cottiaux, D. Delsate, and W. Van Neer, 2018, Aquatic resources in human diet in the Late Mesolithic in Northern France and Luxembourg: insights from carbon, nitrogen and sulphur isotope ratios, Archaeological and Anthropological Sciences, 10, 351-368.

Drucker, D. G., W. Rosendahl, W. Van Neer, M.-J. Weber, I. Görner, and H. Bocherens, 2016, Environment and subsistence in north-western Europe during the Younger Dryas: An isotopic study of the human of Rhünda (Germany), Journal of Archaeological Science: Reports, 6, 690-699.

Dürrwächter, C., O. E. Craig, M. J. Collins, J. Burger, and K. W. Alt, 2006, Beyond the grave: variability in Neolithic diets in Southern Germany?, Journal of Archaeological Science, 33(1), 39-48.

Drucker, D., and H. Bocherens, 2004, Carbon and nitrogen stable isotopes as tracers of change in diet breadth during Middle and Upper Palaeolithic in Europe, International Journal of Osteoarchaeology, 14(3-4), 162-177.

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Meadows, J., H. K. Robson, D. Groß, C. Hegge, H. Lübke, U. Schmöleke, T. Terberger, and B. Gramsch, 2018, How fishy was the inland Mesolithic? New data from Friesack, Brandenburg, Germany, Radiocarbon, 60(5), 1621-1636.

Fernandes, R., P. Grootes, M.-J. Nadeau, and O. Nehlich, 2015, Quantitative diet reconstruction of a Neolithic population using a Bayesian mixing model (FRUITS): The case study of Ostorf (Germany), American Journal of Physical Anthropology, n/a-n/a.

Itahashi Y, Erdal YS, Tekin H, Omar L, Miyake Y, Chikaraishi Y, Ohkouchi N, and Yoneda M. 2019. Amino acid 15N analysis reveals change in the importance of freshwater resources between the hunter-gatherer and farmer in the Neolithic upper Tigris. American Journal of Physical Anthropology 168(4):676-686.

Naito, Y. I., Y. Chikaraishi, N. Ohkouchi, D. Drucker, and H. Bocherens, 2013, Nitrogen isotopic composition of collagen amino acids as an indicator of aquatic resource consumption: insights from Mesolithic and Epipalaeolithic archaeological sites in France, World Archaeology, 45(3), 338-359.













**Table S5: Archaeological and isotopic data of plant samples considered as baseline for the FRUITS models, from four Neolithic German sites (Styring et al. 2017). Data in grey is not considered as baseline in the models.**

- Styring, A., M. Rösch, E. Stephan, H.-P. Stika, E. Fischer, M. Sillmann, and A. Bogaard, 2017, Centralisation and long-term change in farming regimes: Comparing agricultural practices in Neolithic and Iron Age south-west Germany, Proceedings of the Prehistoric Society, 83, 357-381.
- Styring, A. K., R. A. Fraser, R.-M. Arbogast, P. Halstead, V. Isaakidou, J. A. Pearson, M. Schäfer, S. Triantaphyllou, S. M. Valamoti, M. Wallace, A. Bogaard, and R. P. Evershed, 2015, Refining human palaeodietary reconstruction using amino acid  $\delta^{15}\text{N}$  values of plants, animals and humans, Journal of Archaeological Science, 53, 504-515.
- Styring, A., R. Fraser, A. Bogaard, and R. Evershed, 2014, The effect of manuring on cereal and pulse amino acid  $\delta^{15}\text{N}$  values, Phytochemistry, 102.
- Itahashi, Y., Y. S. Erdal, H. Tekin, L. Omar, Y. Miyake, Y. Chikaraishi, N. Ohkouchi, and M. Yoneda, 2019, Amino acid  $\delta^{15}\text{N}$  analysis reveals change in the importance of freshwater resources between the hunter-gatherer and farmer in the Neolithic upper Tigris, American Journal of Physical Anthropology, 168(4), 676-686.

		$\delta^{13}\text{C}$ (‰)				$\delta^{15}\text{N}$ (‰)				Ref	
	N	min	max	Mean	sd	min	max	Mean	sd		
cereals	Vaihingen	13	-24.9	-23.5	<b>-24.2</b>	0.5	4.2	5.7	<b>5.1</b>	0.5	Styring et al. 2017
	Hornstaad Hörnle IA	179	-27.9	-22.9	<b>-25.5</b>	0.9	2.0	11.0	<b>5.0</b>	1.0	Styring et al. 2017
	Sipplingen	44	-27.5	-24.3	<b>-25.7</b>	0.8	3.0	7.2	<b>5.0</b>	1.0	Styring et al. 2017
	Viesenhäuser Hof (Neolithic)	16	-24.3	-22.5	<b>-23.5</b>	0.5	5.4	8.5	<b>6.2</b>	0.8	Styring et al. 2017
	Mean*	252		<b>-24.7</b>	0.7			<b>5.3</b>	0.8		
pulses	Vaihingen	3	-24.1	-23.2	<b>-23.7</b>	0.5	1.8	4.5	<b>3.5</b>	1.5	Styring et al. 2017
	Sipplingen	1	-25.7	-25.7	<b>-25.7</b>		4.7	4.7	<b>4.7</b>		Styring et al. 2017
	Viesenhäuser Hof (Neolithic)	1	-23.5	-23.5	<b>-23.5</b>		2.9	2.9	<b>2.9</b>		Styring et al. 2017
	Mean*	5		<b>-24.3</b>	0.5			<b>3.7</b>	1.5		
<b>Mean* cereals + pulses</b>		<b>257</b>		<b>-24.5</b>	<b>0.6</b>			<b>4.5</b>	<b>1.1</b>		

n	estimate			estimate	
	$\delta^{15}\text{Nb}\text{ulk}$ (‰)	sd	$\delta^{15}\text{NGl}\text{u}$ (‰)	$\delta^{15}\text{NPhe}$ (‰)	
13	<b>4.5</b>	0.5		4	<b>10.8</b>
3	<b>2.8</b>	1.5			

\*mean of the means

**Table S6: Summary of plants isotopic data considered as baseline for the FRUITS models, from four Neolithic German sites (Styring et al. 2015, 2017).**

Styring, A., M. Rösch, E. Stephan, H.-P. Stika, E. Fischer, M. Sillmann, and A. Bogaard, 2017, Centralisation and long-term change in farming regimes: Comparing agricultural practices in Neolithic and Iron Age southwest Germany, Proceedings of the Prehistoric Society, 83, 357-381.

Styring, A. K., R. A. Fraser, R.-M. Arbogast, P. Halstead, V. Isaakidou, J. A. Pearson, M. Schäfer, S. Triantaphyllou, S. M. Valamoti, M. Wallace, A. Bogaard, and R. P. Evershed, 2015, Refining human palaeodietary reconstruction using amino acid  $\delta^{15}\text{N}$  values of plants, animals and humans, Journal of Archaeological Science, 53, 504-515.

Table S7

Food	food category	FDC ID	water	protein	lipid	carbo-hydrate	ash	others (vitamines & mnls)	Glu	Phe	%prot			Mean %prot	Mean %Glu	Mean %Phe	%prot				
											-water)	%Glu	%Phe				food type	range tested	%prot moy	%Glu moy	%Phe moy
pike, steamed or poached	Fish	1098935	72.66	24.22	0.87	0					88.6										
Tuna, fresh, dried	fish	1099038	18.84	76.25							94.0										
Eel, steamed or poached	Fish	1098827	59.26	23.19	14.66	0					56.9						77.8	12.6	3.3		
Lentils, sprouted, raw	Vegetables and Vegetable Products	168427	67.34	8.96	0.55	22.14	1		1.258	0.442	28.3	4.0	1.4								
Lentils, from dried, no added fat	Beans, peas, legumes	1100440	69.23	8.97							29.2										
Lentils, sprouted, cooked, stir-fried, with salt	Vegetables and Vegetable Products	168551	68.7	8.8					1.235	0.434	28.1	3.9	1.4	28.5	4.0	1.4					
Barley, hulled	Cereal Grains and Pasta	170283	9.44	12.48	2.3	73.48			3.261	0.7	13.8	3.6	0.8								
Barley, pearled, cooked	Cereal Grains and Pasta	170285	68.8	2.26					0.591	0.127	7.2	1.9	0.4								
Barley, no added fat	grains	1101534	68.5	2.25							7.1										
Bread, barley	Yeast breads	1100821	35.25	10.67							16.5										
Bread, wheat or cracked wheat	Yeast breads	1100751	35.25	10.67																	
Beer, higher alcohol	Beer	1104397	91	0.9							10.0			10.9	2.7	0.6					
Wheat, durum	Cereal Grains and Pasta	169721	10.94	13.68	2.47	71.13	1.78		4.743	0.681	15.7	5.4	0.8								
Wheat, sprouted	Cereal Grains and Pasta	169725	47.75	7.49	1.27	42.53	0.96		1.871	0.35	14.6	3.6	0.7								
Bread, pita, whole wheat	Yeast breads	1100761	30.6	9.8	1.71	55.89					14.1										
Whole wheat cereal, cooked, no added fat	Grits and other cooked cereals	1101681	87.27	1.55							12.2										
Cereals, whole wheat hot natural cereal, cooked with water, with salt	Breakfast Cereals	171677	83.6	2							12.2			13.8	4.5	0.7					

Table S7: Example of composition (nutrient fraction concentrations) of different food items and range of values considered for FRUITS models, from USDA database (Haytowitz et al., 2019)

Haytowitz, D. B., J. K. C. Ahuja, X. Wu, M. Somanchi, M. Nickle, Q. A. Nguyen, J. M. Roseland, J. R. Williams, K. Y. Patterson, Y. Li, and P. R. Pehrsson, 2019, USDA National Nutrient Database for Standard Reference, Legacy Release. Nutrient Data Laboratory, Beltsville Human Nutrition Research Center, ARS, USDA. <https://data.nal.usda.gov/dataset/usda-national-nutrient-database-standard-reference-legacy-release>. Accessed 2021-02-26.

## Data S1

### FRUITS models, baseline reference and parameters

For comparison purposes with other studies and with the aim of broadening the discussion, we tried to model our entire isotope data set ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $\delta^{34}\text{S}$  of bulk collagen and  $\delta^{15}\text{N}_{\text{AA}}$ ). The **limitations** of the use of such models are discussed and the results are merely provided as examples but do not reflect a definitive interpretative result. Indeed, the result of the models strongly depends on the input parameters which are often unknown or uncertain, and it is worth considering that these kinds of models are perhaps simply not suitable for archaeology. However, their application offers a picture easily comparable to other contexts and above all comparable between individuals in a single context, insofar as the input parameters used are the most appropriate ones, i.e. giving priority to probability over precision and using comparable settings. Whereas all models are approximately equal (Cheung and Szpak, 2020), we chose the most appropriate for us and the most widely used in similar contexts (European Neolithic), namely the Bayesian mixing model **FRUITS** (Food Reconstruction Using Isotopic Transferred Signals; v3.0 Beta; Fernandes et al., 2014). Therefore, all the settings inherent to the methodology are following Fernandes et al. (2014) and are described in **Tables S8-S10**.

Considering the **food sources**, the models run for GLN humans are based on the Yonne baseline, composed of domestic isotopic values (wild animals are not considered in these models) from several Neolithic sites located in the Yonne valley, which represent a sufficient and consistent dataset for the animal end-member. For the NFN human, the models are based on the Yonne + LV + NFN baseline (animal values from both the Yonne valley and the LV and NFN sites), as the NFN dataset alone does not provide enough data to constitute a reliable baseline. A database is given for each baseline in **Tables S1-S7** and a mean value of each food source is plotted on the graphs (**Fig. 2** and **Fig. 4**). While the values of **local and contemporary terrestrial animals** were measured directly along with humans, no plant or fish remains were found on the sites. Archaeoichthyological and archaeobotanical data are besides very scarce in the region and the consumption/production/management of these resources is rather unknown. Consequently, the plants and freshwater baselines are taken from the literature (**Tables S4-S6**).

For **plants**, the data available for German Neolithic sites (Styring et al., 2017) were used. Mediterranean and further North sites have a relatively different climate and environment that may impact the isotopic ratios (Craine et al., 2009; Gavériaux et al., accepted; Goude and Fontugne, 2016). They have therefore not been used as baseline here. Earlier sites do not yield cereals, recently cultivated by early farmers, and more recent sites may be more subject to more intense agricultural practices (intensive manuring and water management; e.g. Varalli et al., 2021), so they are also excluded here. As it has been shown that humans did not consume the same plants as herbivores (Bogaard et al., 2017), we preferred to use, for C and N isotopes, plant values measured in other sites from a similar context (Neolithic Germany; Styring et al., 2017) rather than our local herbivore values subtracting the trophic enrichment (this gives the forage values). Contrarily, for S, plant values have been estimated from domestic fauna, as suggested by Fernandes et al. (2015), since S isotopic ratio is highly variable with environment (Nehlich, 2015) and there is no fractionation between prey and consumer (Nehlich, 2015; Tanz and Schmidt, 2010). For AA, we used the  $\delta^{15}\text{N}_{\text{Glu}}$  value estimated by Styring et al. (2015) for the German Neolithic site of Vaihingen. As archaeological plants do not contain sufficient AA content, the  $\delta^{15}\text{N}_{\text{Glu}}$  value is estimated from comparison of their measured  $\delta^{15}\text{N}_{\text{bulk}}$  and known difference between  $\delta^{15}\text{N}_{\text{bulk}}$  and  $\delta^{15}\text{N}_{\text{Glu}}$  in modern cereal grains. In this case, this means subtracting 0.5‰ from the  $\delta^{15}\text{N}_{\text{bulk}}$  values of charred grains from Vaihingen. This gives a very rough estimate, for lack of a better way, based on plant values from another context combined to an approximate  $\Delta^{15}\text{N}_{\text{bulk-Glu}}$  value based on modern samples from other contexts. In addition, the plant

$\delta^{15}\text{N}_{\text{Phe}}$  value is estimated from the average human and domestic animal  $\delta^{15}\text{N}_{\text{Phe}}$  value of the samples analyzed in this study minus the theoretical trophic enrichment of 0.4‰ (namely  $\delta^{15}\text{N}_{\text{Phe}} \approx 11.2 (\pm 1.7) - 0.4 \approx 10.8 \pm 1.7$  ‰) according to Styring et al. (2015) recommendations. This value is the same than those estimated for Vaihingen (*ibid*).

For **aquatic resources, namely freshwater fish** (riparian are not considered in this study, since they have never been found in the region for the Neolithic period and they are not supposed to be consumed), we have considered a maximum of data from sites located in neighboring regions and belonging to similar or earlier periods (to avoid any pollution effect occurring later). All the data is given in [Table S4](#) and the whole variability is highlighted in [Figure S1](#).

To these values are added the corresponding **offsets** for each fraction (between macronutrient and collagen for animals or between macronutrient and bulk sample for plants), following the Fernandes et al. (2014) guidelines (all details in [Tables S8-S10](#)). In addition, the offsets between diet and consumer are given for each proxy along with the corresponding weight contribution of each fraction according to routing metabolism ([Tables S8-S10](#)). These offsets, also called trophic enrichment factor (TEF), are known to be variable depending on the species and the diet (Arneson and MacAvoy, 2005; Barnes and Jennings, 2007; Blanke et al., 2017; Bocherens and Drucker, 2003; Chikaraishi et al., 2015; McCutchan et al., 2003; Minagawa and Wada, 1984). For the sake of consistency, we chose to apply those recommended by Fernandes et al. (2014) for stable isotopes in bulk collagen and by Chikaraishi et al. (2009) and Steffan et al. (2013) for amino acids.

Finally, the **concentrations** of each food source for each fraction were assessed from USDA (Haytowitz et al., 2019), according to the food item considered in the studied context ([Table S7](#)). These values are close to those of other studies in similar context (e.g. Fernandes et al., 2015; Meadows et al., 2016). Since these concentrations can be variable according to the food, we try different models with: plant concentration between 10 and 15% of protein, meat from 30 to 80% of protein depending on the meat species (pig more fatty than herbivores) and the pieces (muscle vs offal), fish from 70 to 80% of protein. Regarding the **milk** consumption issue, it is not possible to distinguish animal proteins from milk or meat through bone collagen analysis. Nevertheless, the more lipid-rich composition of milk can be taken into account in the models, by varying the concentration of the “meat” source (given for “animal proteins”). However, varying these concentration values does not substantially affect the results here.

## RESULTS AND DISCUSSION

Because of too many uncertainties (variable offsets, TDF and  $\beta$  value, unknown local food source values and food sources themselves, variable concentrations), the models are not expected to represent the true proportion of foodstuffs in the diet. They can only give some insights easily comparable to other sites/contexts where the same modeling has been applied, with the caveat that they can be quite different depending on the settings involved. In particular, this kind of models can be useful to further discuss the inter-individual variability.

Several tests have been performed by varying the input options to assess the variability of the model results. Food sources and their values constitute the most contingent/determining parameters to varying the outputs; other settings (concentrations, offsets, weights) are a little less sensitive. For the sake of consistency, similar food sources and corresponding values to those already published were chosen, namely for plants and fish. The [Figure S3](#) presents one example of outputs that appeared to be the most relevant for all the models based on C, N and S analysis of bulk collagen, CSIA-AA and all proxies combined for both GLN and NFN samples separately. All input parameters involved, applied

offsets for each food category and proxy and all food values by dietary fraction (protein, energy, glutamic acid and phenylalanine) are given in Tables S8-S10. Since FRUITS is optimal for n proxies at n+1 sources, we voluntarily chose not to consider separately cereals from pulses or other plant species, on the one hand, and to exclude wild herbivores, on the other hand. In order not to multiply the uncertainties, we only retained the same four sources in the third combined model.

Overall, the models reveal more plant intake than assumed for a pure carnivorous diet. However, plant values considered here have a TP slightly higher than 1 and this must be kept in mind for the following interpretations. The GluPhe models at 3 sources (B, C, F) show lower plant contribution than models including CNS isotopic values of bulk collagen, according to our interpretations from the raw data. Moreover, GluPhe models highlight, on the one hand, greater pig consumption (model C) than bulk model A, confirming pig was the secondary resource after cattle and, on the other hand, less fish in the diet (models B and F).

The **GluPhe models at 3 sources** (B and C) suggest a lower plant contribution (approximately from 30 to 60%) than the CNS model, more domestic animals (approximately from 30 to 60%) of which more pig (approximately between 10 and 40%) and less, or as much, fish (<10%, in average lower than with the CNS model).

The condensed **model CNS-GluPhe** at 4 sources (models D and G; Figure S3) corresponds to a mixture of the two separate models (CNS and GluPhe) and highlights even less fish in diet (<5% fish).

For **Poncharaud (NFN)**, a separated model was processed, based on a different baseline, and provides a rather similar result to that of GLN 284 (Figure S3), in agreement with what is observed with the raw data: approximately between 40 and 80% of plants (GluPhe model F) or more than 80% (CNS model E), less than 60% of domestic animals (GluPhe model F) or less than 20% (CNS model E) and very few or no fish with both models. The combined CNS-GluPhe model G is close to the CNS one with even less fish intake.

All the model results bring interesting information regarding the plant consumption, potentially previously underestimated, although considering that plants' TP considered here is higher than 1. According to these models, the consumption of fish was negligible (<5%), which is in line with our interpretation without models. These estimations (plants underestimated and fish <5%) are consistent with a recent study of European Neolithic sites (Bickle, 2018). However, the plant contribution estimated at GLN is still significantly low (close to 40-60% excluding the outlier GLN284) and specifically lower than those estimated by A. Bickle. This supports a particularly high trophic level of these humans which rely more on animal resources. This also supports the assumption on the variability even within the Neolithic, between groups, with the diet making social cohesion: "shared dietary practices were a strong part of community cohesion for early agricultural communities" (Bickle, 2018). This particularly supports a regional specificity already highlighted by several parameters as funerary practices, grave goods or diet (Chambon et al., 2013; Rey et al., 2019).

Several uncertainties do complicate the analysis: what percentage of meat vs. milk should be deemed? What about cereal manuring? What percentage of pulses or wild plants or other resources? Indeed numerous other resources could be consumed, which leave no trace over time and whose isotopic signature is not known, as for example tuber, fruits, wild herbs (some protein-rich as nettle), legumes, mollusks, eggs, etc. In any case, models are interesting to compare individuals from a single context (see later).

For comparison purposes, FRUITS model have been applied to Neolithic people from Lake Ostorf in Germany where it confirms the significant consumption of proteins from fish in addition to terrestrial animal proteins and plants for calories (Fernandes et al., 2015). In a closer context of Early Neolithic in Alsace, FRUITS results highlighted that crops were very predominant while fish is not significant

(Denaire et al., 2017). To broaden the comparison, FRUITS model applied to Bronze Age people from Eastern Europe shows a probably more terrestrial subsistence strategy than previous hunter-gatherers, although freshwater resources are not always excluded from the diet (Shishlina et al., 2016; Tõrv and Meadows, 2015). On the contrary, a much larger crops intake is revealed for Bronze Age Swiss people where fish is not even considered (Knipper et al., 2020). In comparison with all these examples, our results reveal once again the weak probability to consume freshwater resources at GLN and NFN and the particularly high trophic level for most humans from GLN, signing a quite carnivorous diet.

One advantage of FRUITS model is primarily to **combine all the proxies into a single model**, which has never been done to our knowledge (Figure S3, D and H), and thus to assess the overall inter-individual variability. **No sex-related distinction** is highlighted, in agreement with the observation based on the TP graph (Fig. 4). While slight sex-related differences were highlighted for C, N and S isotope ratios on bulk collagen in the whole GLN sample (Rey et al., 2019), here the sample size is too small and unbalanced. Sexual differences are not visible, neither in the CNS models nor in the GluPhe models and graph. Only note that the highest  $\delta^{15}\text{N}_{\text{bulk}}$  values do not correspond to the highest TP<sub>AA</sub>.

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**Table S8:** Summary of input parameters for FRUITS models.

Proxies	Offset	unc.	protein weight	unc.	energy weight	unc.	Phe weight	unc.
d13C	<b>4.8</b>	0.2	<b>74</b>	4	<b>26</b>	4	<b>0</b>	
d15N	<b>5.5</b>	0.5	<b>100</b>	0	<b>0</b>		<b>0</b>	
d34S	<b>0.5</b>	0.5	<b>100</b>	0	<b>0</b>		<b>0</b>	
d15NGlu	<b>8.0</b>	1.1	<b>100</b>	0	<b>0</b>		<b>0</b>	
d15NPhe	<b>0.4</b>	0.4	<b>0</b>	0	<b>0</b>		<b>100</b>	0

Concentrations (variable)	protein	unc.	energy	unc.	Phe	unc.
plant	<b>15</b>	2.5	<b>85</b>	2.5	<b>0.8</b>	0.1
dom animals (herbiv + pig)	<b>50</b>	5	<b>50</b>	5	<b>2.4</b>	0.5
dom herbiv	<b>60</b>	5	<b>40</b>	5	<b>2.6</b>	0.5
pig (GLN)	<b>50</b>	5	<b>50</b>	5	<b>2.2</b>	0.5
fish (Europe)	<b>70</b>	5	<b>30</b>	5	<b>3.3</b>	0.5

**Table S9:** Summary of offsets for FRUITS models.

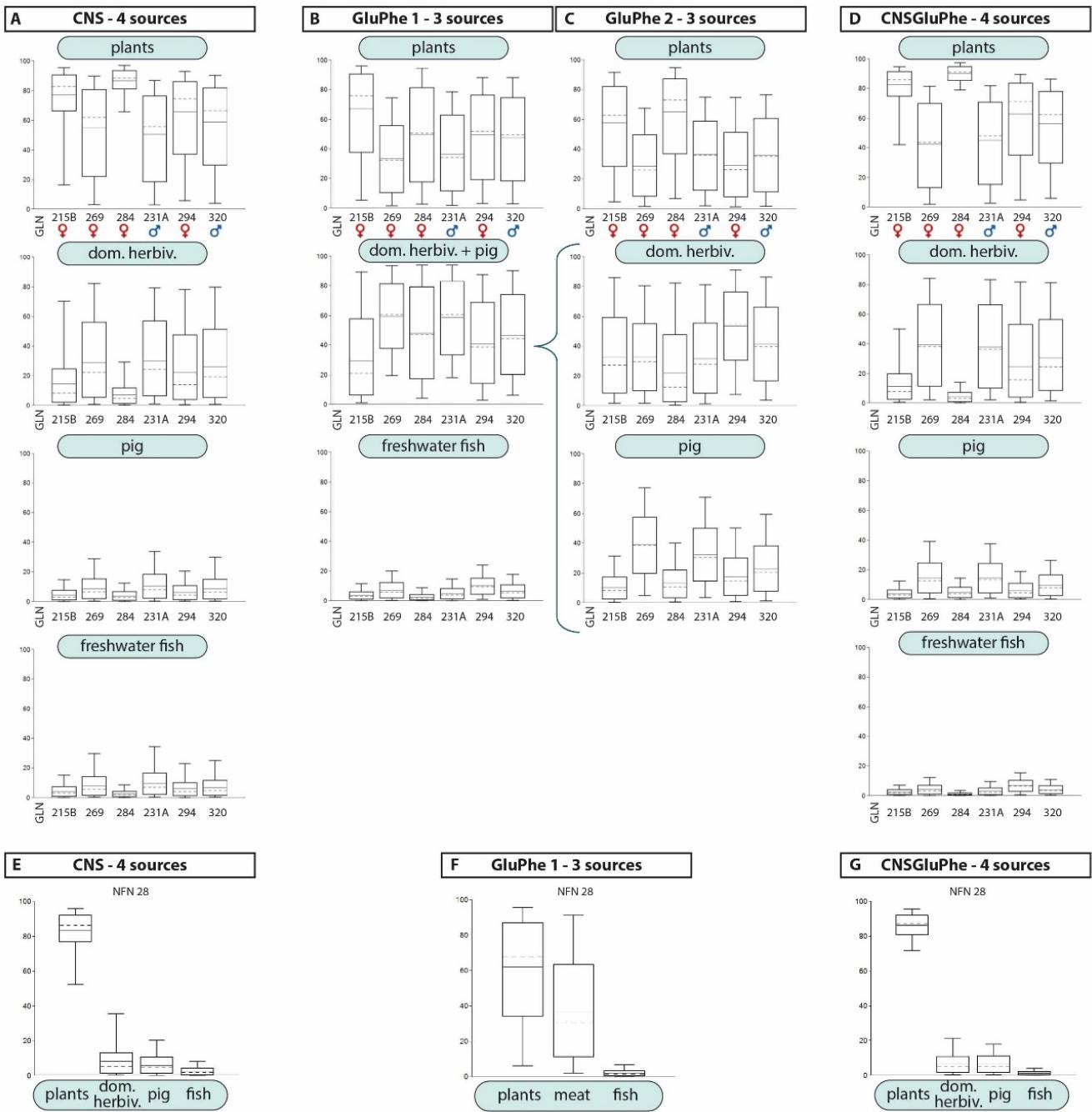
Offsets	<i>Fernandes et al. 2015</i>		
<b>for protein fraction:</b>			
$\Delta^{13}\text{C}_{\text{bulk-prot}}$ plants =	<b>-2</b>	$\Delta^{15}\text{N}_{\text{bulk-prot}}$ plants =	<b>0</b>
$\Delta^{13}\text{C}_{\text{prot-col}}$ ter. anls =	<b>-2</b>	$\Delta^{15}\text{N}_{\text{prot-col}}$ ter. Anls =	<b>+2</b>
$\Delta^{13}\text{C}_{\text{prot-col}}$ fish =	<b>-1</b>	$\Delta^{15}\text{N}_{\text{prot-col}}$ fish =	<b>+2</b>
<b>for energy fraction:</b>			
$\Delta^{13}\text{C}_{\text{bulk-carbs}}$ plants =	<b>+0.5</b>		
$\Delta^{13}\text{C}_{\text{lipids-col}}$ ter. anls =	<b>-8</b>		
$\Delta^{13}\text{C}_{\text{lipids-col}}$ fish =	<b>-7</b>		
<b>Offsets diet-consumer</b>			
	<b>%o</b>		unc
$\Delta^{13}\text{C}_{\text{diet-consumer col}}$	<b>4.8</b>		0.2
$\Delta^{15}\text{N}_{\text{diet-consumer col}}$	<b>5.5</b>		0.5
$\Delta^{34}\text{S}_{\text{diet-consumer col}}$	<b>0.5</b>		0.5
$\Delta^{15}\text{NGlu}_{\text{diet-consumer col}}$	<b>8.0</b>		1.1
$\Delta^{15}\text{NPh}$ <sub>diet-consumer col</sub>	<b>0.4</b>		0.4

+ uncertainty of 0.5 %o for each indiv

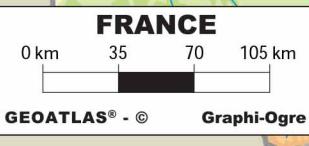
+ uncertainty of 1 %o for each source

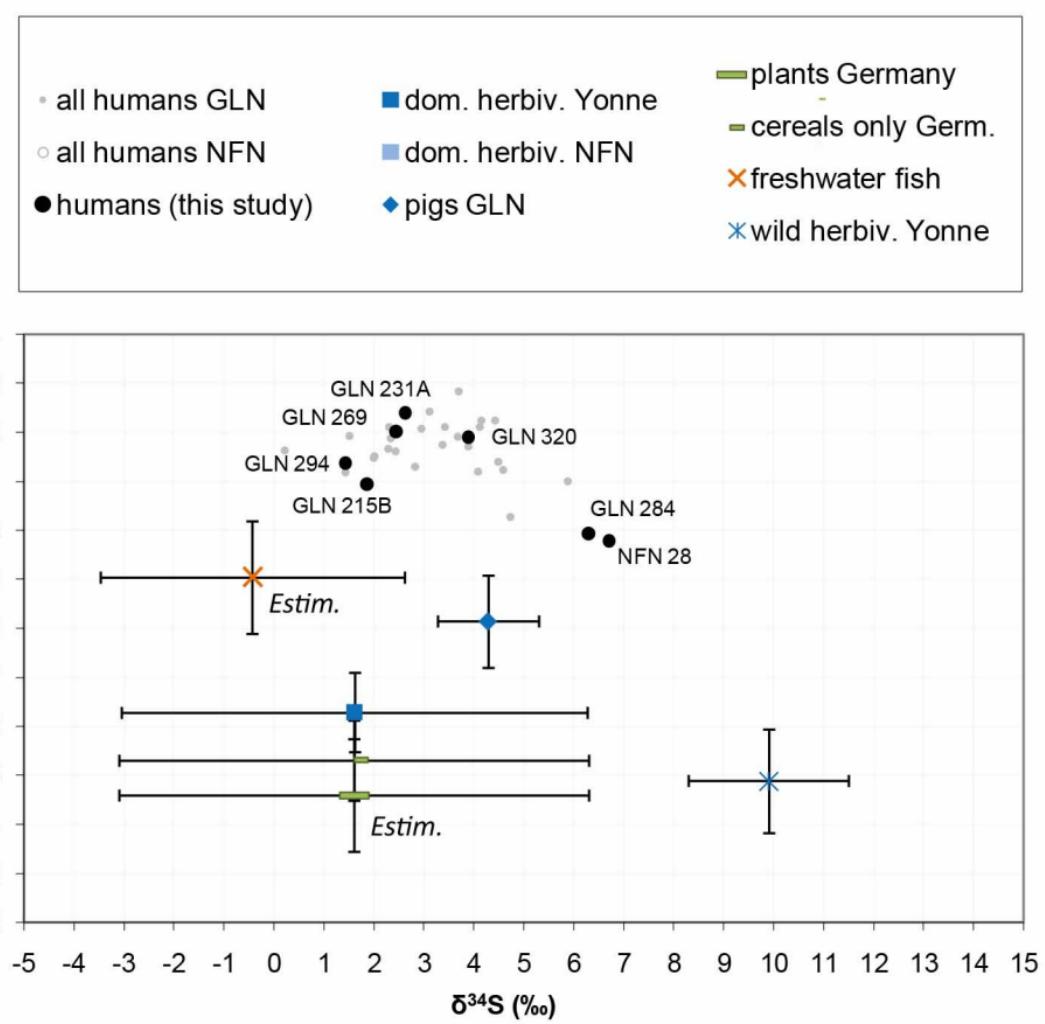
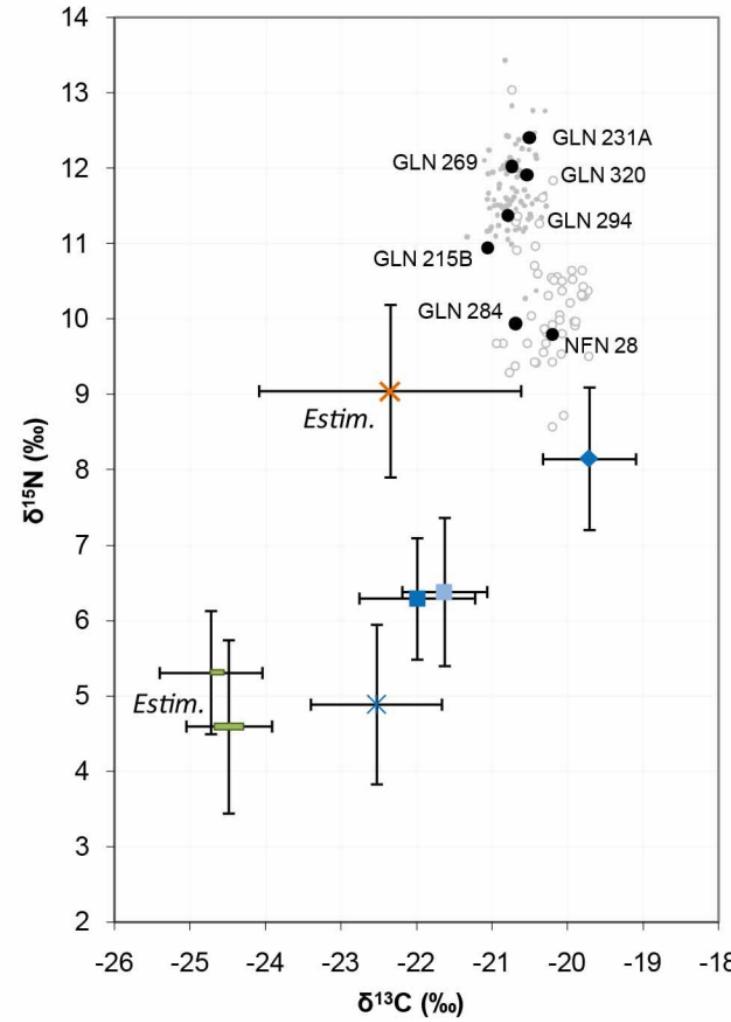
**Table S10:** Summary of food values for FRUITS models.

	$\delta^{13}\text{C}$	unc.	$\delta^{15}\text{N}$	unc.	$\delta^{34}\text{S}$	unc.	$\delta^{15}\text{N}_{\text{Glu}}$	unc.	$\delta^{15}\text{N}_{\text{Phe}}$	unc.
raw values + offsets										
plant prot	<b>-26.5</b>	0.6	<b>4.6</b>	1.1	<b>1.6</b>	4.7	<b>4.0</b>	1.0		
plant energy	<b>-24.0</b>	0.6								
plant Phe							<b>10.8</b>	1.0		
cereals prot	<b>-26.7</b>	0.7	<b>5.3</b>	0.8	<b>1.6</b>	4.7	<b>5.4</b>	0.9		not used in these models
cereals energy	<b>-24.2</b>	0.7								
cereals Phe							<b>10.8</b>	1.0		
pulses prot	<b>-26.3</b>	0.5	<b>3.7</b>	1.5	<b>1.6</b>	4.7	<b>4.3</b>	1.3		not used in these models
pulses energy	<b>-23.8</b>	0.5								
pulses Phe							<b>10.8</b>	1.0		
dom animal prot (total mean)	<b>-23.6</b>	1.1	<b>8.6</b>	1.1	<b>2.3</b>	4.2	<b>10.6</b>	2.2		
dom animal energy (total mean)	<b>-29.6</b>	1.1								
dom animal Phe (total mean)							<b>10.9</b>	1.7		
dom herbiv prot	<b>-24.0</b>	0.8	<b>8.3</b>	0.8	<b>1.6</b>	4.7	<b>9.4</b>	1.2		
dom herbiv energy	<b>-30.0</b>	0.8								
dom herbiv Phe							<b>10.0</b>	1.1		
pig GLN prot	<b>-21.7</b>	0.6	<b>10.2</b>	0.9	<b>4.3</b>	1.0	<b>12.9</b>	0.0		
pig GLN energy	<b>-27.7</b>	0.6								
pig GLN Phe							<b>12.6</b>	0.0		
wild herbiv prot	<b>-24.5</b>	0.9	<b>6.9</b>	1.1	<b>9.9</b>	1.6	<b>7.0</b>	2.5		not used in these models
wild herbiv energy	<b>-30.5</b>	0.9								
wild herbiv Phe							<b>7.9</b>	2.7		
freshwater fish prot	<b>-23.3</b>	1.7	<b>11.0</b>	1.1	<b>-0.4</b>	3.0	<b>20.7</b>	1.2		
freshwater fish energy	<b>-29.3</b>	1.7								
freshwater fish Phe							<b>4.5</b>	1.7		
<b>dom animal Yonne+Pch+LV (total mean)</b>										
dom animal prot	<b>-23.4</b>	1.1	<b>8.8</b>	1.2	<b>6.6</b>	6.5	<b>10.3</b>	1.4		
dom animal energy	<b>-29.4</b>	1.1								
dom animal Phe							<b>11.1</b>	1.1		
herbiv dom prot	<b>-23.7</b>	0.8	<b>8.5</b>	0.8	<b>7.1</b>	4.7	<b>9.7</b>	1.2		
herbiv dom energy	<b>-29.7</b>									
herbiv dom Phe							<b>11.0</b>	1.1		
pig (Gurgy+Pch) prot	<b>-22.2</b>	0.6	<b>9.0</b>	0.9	<b>4.3</b>	1.0	<b>12.9</b>	0.0		
pig (Gurgy+Pch) energy	<b>-28.2</b>									
pig (Gurgy+Pch) Phe							<b>12.6</b>	0.0		



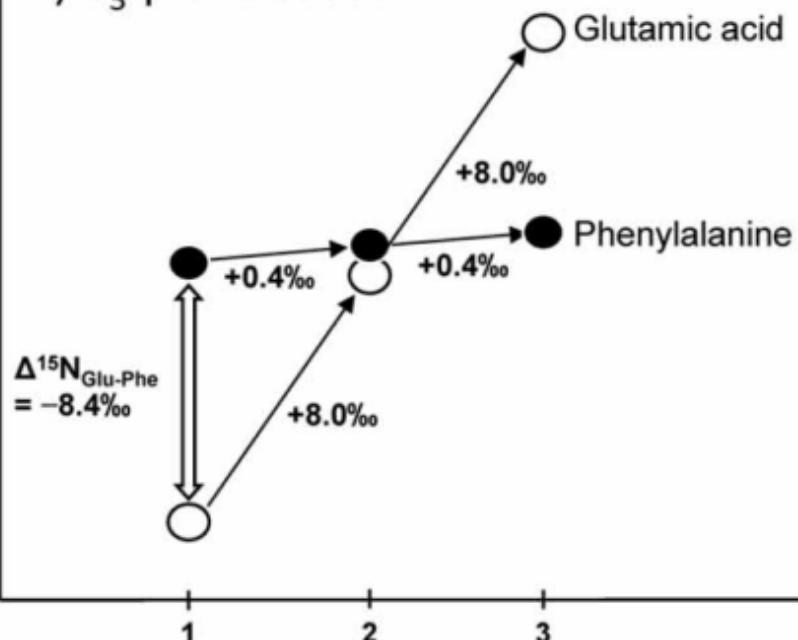
**Figure S3:** Model estimates of the relative contribution in the diet of GLN and NFN individuals of different food sources considered under specific conditions determined by the input parameters given in SI\_models. Models are based on C, N and S isotope ratios of bulk collagen (A and E); N isotope ratios of Glu and Phe for 3 different sources (B-C and F); all proxies combined C, N and S isotope ratios of bulk collagen and N isotope ratios of Glu and Phe (D and G), for individuals from GLN (A-D) and NFN (E-G). Boxes represent a 68% credible interval (corresponding to the 16th and 84th percentiles) while the whiskers represent a 95% credible interval (corresponding to the 2.5th and 97.5th percentiles). The horizontal continuous line represents the estimated mean while the horizontal discontinuous line represents the estimated median (50th percentile).





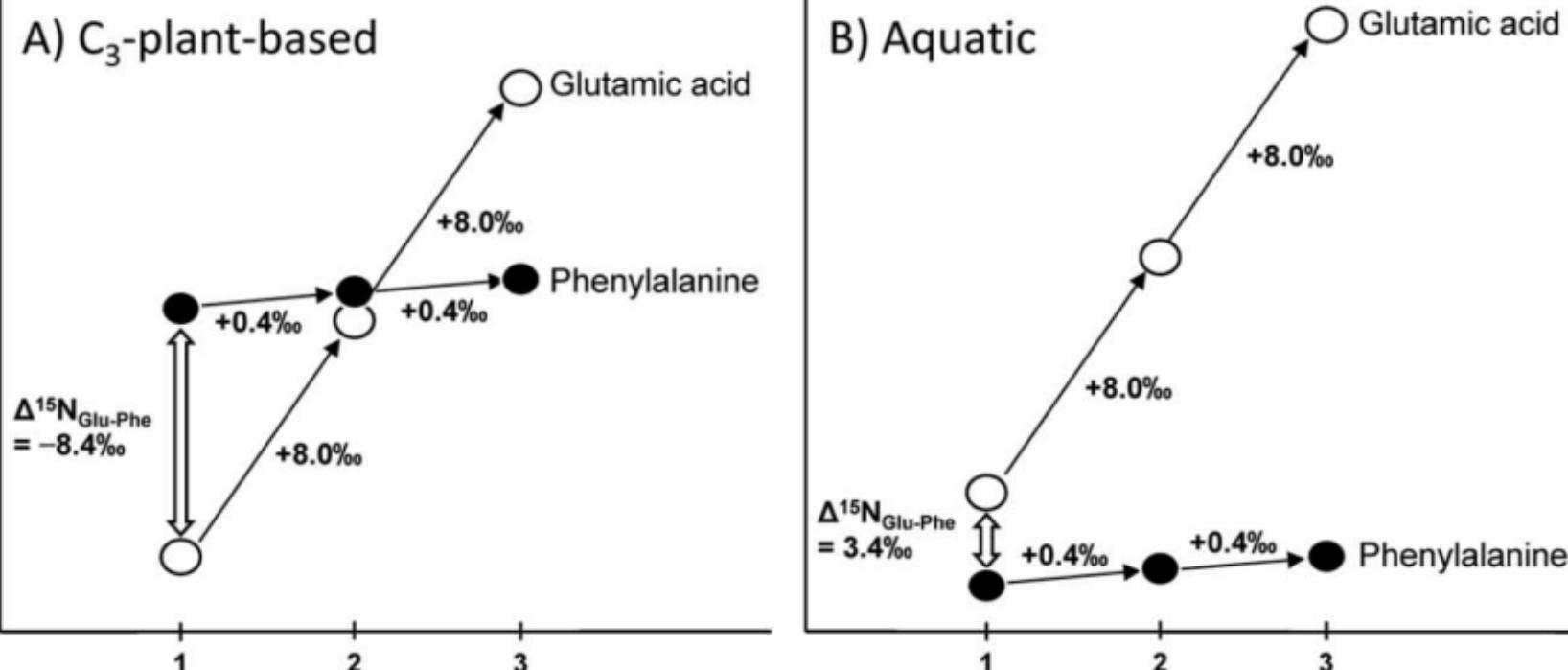
A) C<sub>3</sub>-plant-based

$\delta^{15}\text{N}$  (‰)

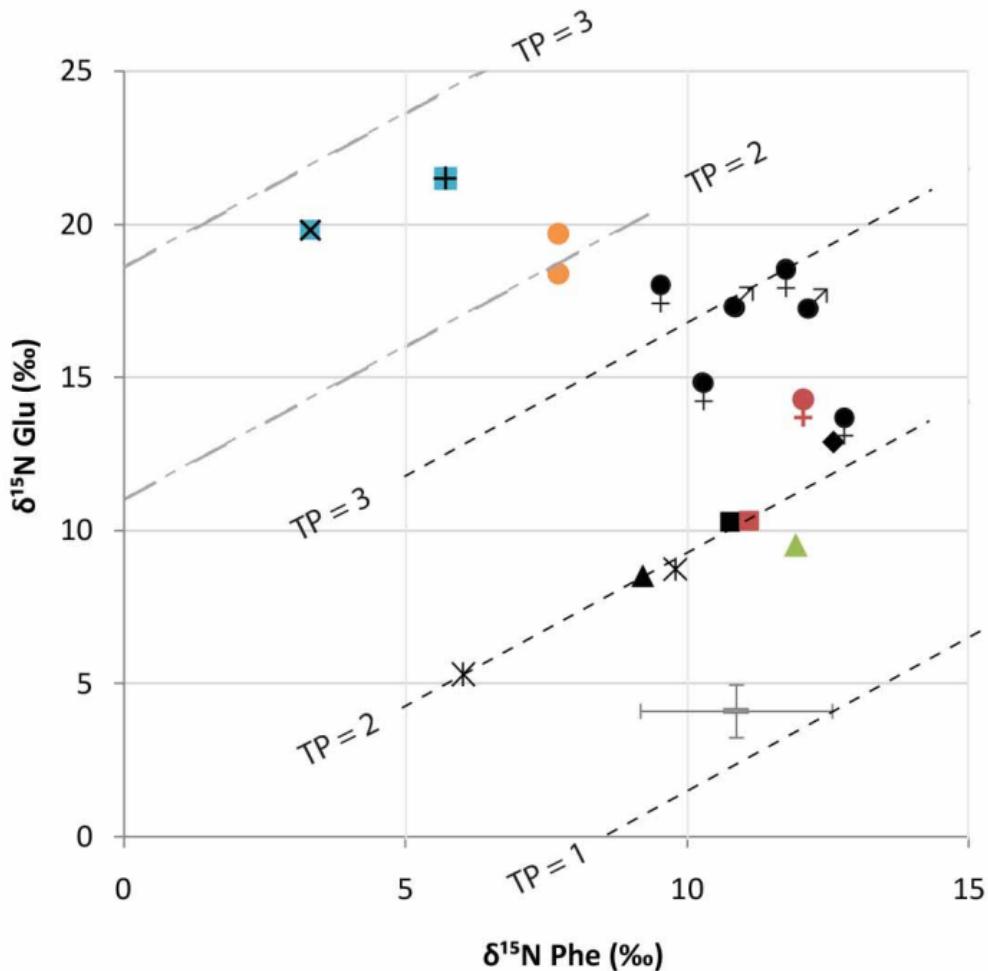


Trophic Position (C<sub>3</sub>)

B) Aquatic



Trophic Position (Aqua)



- GLN, BMT (Yonne)
  - Pontcharaud
  - Le Vigneau
  - Noyen-sur-Seine (Meso)
  - Pont d'Ambon (Meso)
  - human      ♂ male
  - ◆ pig      ♀ female
  - cattle
  - ▲ caprine
  - × deer
  - ⊕ pike
  - ⊗ carp
  - plants estimate
  - - - TP (ter)
  - - - TP (aqua)
- this study

Archaeological site	ID	Archaeological ID	Common name	Species	Sex	Age at death	Analyzed bone	Archaeological reference	CNS data reference
Beaumont "Le Crot aux Moines"	BMT F10	Merlange A2.5	caprine	(Caprinae family)		adult	long bone	1	7
Beaumont "Le Crot aux Moines"	BMT F14	Merlange D4.9.X2	red deer	<i>Cervus elaphus</i>		indet	talus	1	7
Gurgy "Les Noisats"	GLN 215B	st 215B	human	<i>Homo sapiens</i>	F*	8-14	long bone	2, 3, 4	7, 8
Gurgy "Les Noisats"	GLN 231A	st 231A	human	<i>Homo sapiens</i>	M	>20	long bone	2, 3, 4	7, 8
Gurgy "Les Noisats"	GLN 269	st 269	human	<i>Homo sapiens</i>	F	>20	long bone	2, 3, 4	7, 8
Gurgy "Les Noisats"	GLN 284	st 284	human	<i>Homo sapiens</i>	F	>20	long bone	2, 3, 4	7
Gurgy "Les Noisats"	GLN 294	st 294	human	<i>Homo sapiens</i>	F*	>20	long bone	2, 3, 4	7, 8
Gurgy "Les Noisats"	GLN 320	st 320	human	<i>Homo sapiens</i>	M	>20	long bone	2, 3, 4	7, 8
Gurgy "Les Noisats"	GLN F8	st 38-14	pig	<i>Sus scrofa domesticus</i>		indet	coxal	2, 3, 4	7
Gurgy "Les Noisats"	GLN F15	st 60-35	cattle	<i>Bos taurus</i>		12-36 months?	long bone	2, 3, 4	7
Gurgy "Les Noisats"	GLN F16	st 60-36	roe deer	<i>Capreolus capreolus</i>		adult	MT	2, 3, 4	7
Le Vigneau	LVF9		sheep	<i>Ovis aries</i>		18 months		2, 3, 4	9
Pontcharaud	NFNA01		cattle	<i>Bos taurus</i>				6	10, 11
Pontcharaud	NFN28	sep 38	human	<i>Homo sapiens</i>	F			6	10, 11

**Table 1: Archaeological and biological data of the sample selected for the CSIA-AA and complementary analytical information.** \*sex determined by genetics (Rivollat, pers. comm.) 1: Chambon et al. 2013, 2: Rivollat et al. 2015, 3: Rottier et al. 2005, 4: Rottier 2007, 5: Coutelas et al. 2015, 6: Loison1998, 7: Rey et al. 2019, 8: Rey et al. 2017, 9: Goude et al. 2019, 10: Goude et al. 2013, 11: Goude et al. 2021

ID	species	$\delta^{13}\text{C}$ bulk	$\delta^{15}\text{N}$ bulk	$\delta^{34}\text{S}$ bulk	ref CNS iso	Ala	Gly	Val	Leu	Ile	Pro	Asp+ Thr	Glu	Phe	Hyp	TP (Ter)	TP (Aqua)	
BMT F10	caprine	-22.3	6.0		1								<b>8.5</b>	<b>9.2</b>		2.0		
BMT F14	red deer	-21.4	5.3		1				9.0	7.4	9.0		<b>8.8</b>	<b>9.8</b>	7.7	2.0		
GLN 215B	human	-21.1	10.9	1.9	1. 2							17.8		<b>14.8</b>	<b>10.3</b>	14.6	2.7	1.2
GLN 231A	human	-20.5	12.4	2.6	1. 2	14.5	11.6	-3.1				21.6	11.8	<b>17.3</b>	<b>12.2</b>	17.8	2.8	1.2
GLN 269	human	-20.7	12.0	2.4	1. 2	13.3	10.4	13.3				19.4	13.7	<b>18.6</b>	<b>11.8</b>	16.6	3.0	1.4
GLN 284	human	-20.7	9.9	6.3	1									<b>13.7</b>	<b>12.8</b>		2.2	0.7
GLN 294	human	-20.8	11.4	1.4	1. 2	14.0	11.4	13.7				18.2	13.1	<b>18.0</b>	<b>9.5</b>	14.3	3.2	1.7
GLN 320	human	-20.5	11.9	3.9	1. 2	14.9	12.5	13.9				21.3	13.8	<b>17.3</b>	<b>10.8</b>	17.3	3.0	1.4
GLN F8	pig	-19.3	8.5		1				11.9	11.7	14.0			<b>12.9</b>	<b>12.6</b>	12.1	2.1	
GLN F15	cattle	-22.6	6.9		1				11.1	12.4	10.5			<b>10.3</b>	<b>10.8</b>	9.1	2.0	
GLN F16	roe deer	-22.8	3.3	11.8	1				5.6	4.3				<b>5.3</b>	<b>6.0</b>	6.5	2.0	
LVF9	sheep	-21.4	6.7	11.5	3									<b>9.5</b>	<b>11.9</b>	9.2	1.8	
NFNA01	cattle	-22.4	8.0	8.3	4. 5				7.0	11.7				<b>10.3</b>	<b>11.1</b>	10.6	2.0	
NFN28	human	-20.2	9.8	6.7	4. 5							16.8		<b>14.3</b>	<b>12.1</b>	14.2	2.4	0.8

**Table 2: Carbon, nitrogen and sulphur isotopic values of bulk collagen, nitrogen isotopic values of single amino acids and corresponding trophic position in terrestrial C3-plant based ecosystem (Ter) and aquatic ecosystem (Aqua). 1: Rey et al. 2019, 2: Rey et al. 2017, 3: Goude et al. 2019, 4: Goude et al. 2013, 5: Goude et al. 2021**