#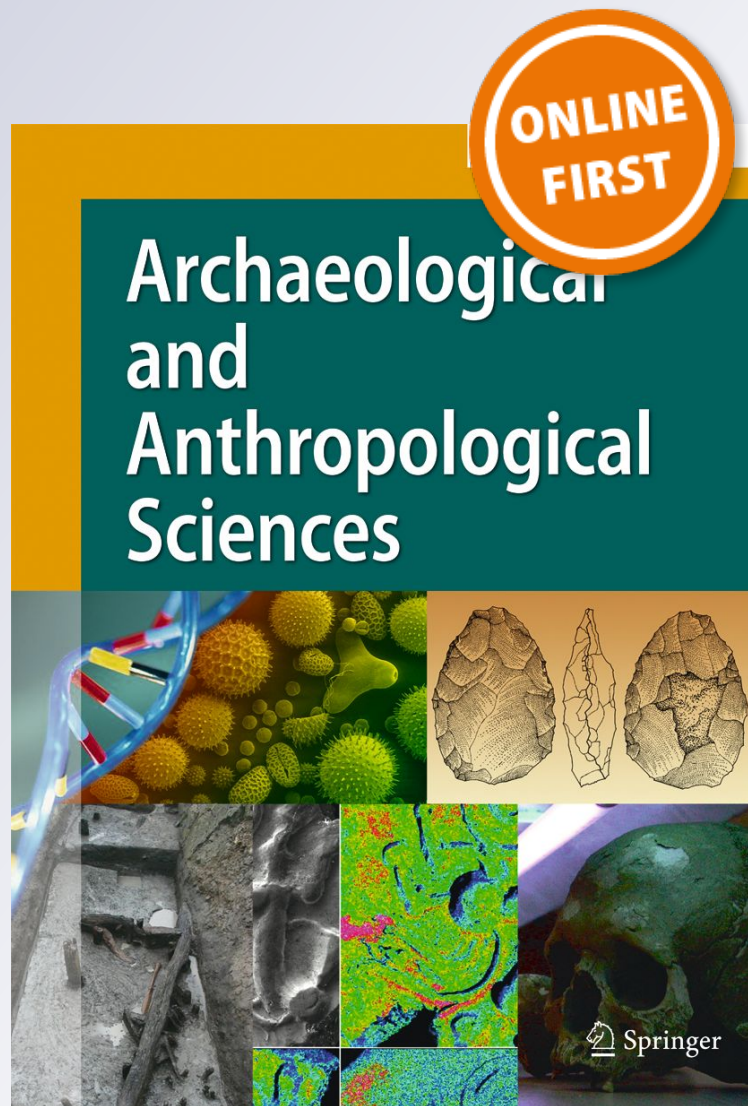 A multi-isotope analysis of Neolithic human groups in the Yonne valley, Northern France: insights into dietary patterns and social structure

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A multi-isotope analysis of Neolithic human groups in the Yonne valley, Northern France: insights into dietary patterns and social structure

L. Rey [1] · Domingo C. Salazar-García [2,3] · P. h. Chambon [4] · F. Santos [1] · S. Rottier [1] · G. Goude [5]

Abstract

With the arrival of the Neolithic to Europe, new ways of life and new subsistence strategies emerged. In the Paris Basin (northern France), the appearance of some monumental funerary structures during the Middle Neolithic highlights in particular the increasing complexity of the social organisation. At the same time, several sites, such as open-air cemeteries, do not display any evidence of such arrangement. In the southeast of this area, the two primary routes of neolithisation meet. Several funerary parameters attest to the diverse influence received from other surrounding cultures. In order to assess potential differences in diet, and therefore on purported social distinctions at the inter- and intra-site level, stable isotope analyses (carbon, nitrogen and sulphur) were performed on bone collagen of humans ($n = 177$) and non-human animals ($n = 62$) from seven archaeological sites located in the same area (< 10 km). This study is the biggest so far on French Neolithic material and thus allows for an extensive investigation at a regional scale. Results show that the human nitrogen isotopic ratios are relatively enriched in nitrogen-15 comparing to those of the domesticated animals. This reflects a trophic step that is rarely observed elsewhere in the surrounding Neolithic people, particularly for humans of the biggest site Gurgy "Les Noisats". Though zooarchaeological data support a predominant cattle consumption, here, we propose a mixed protein consumption of cattle and pig, possibly complemented with some freshwater resources. Furthermore, carbon, nitrogen and sulphur isotopic ratios suggest some slight differences between sexes and sites. This sexual distinction has rarely been identified in the diet within a Neolithic context. Some variations over time were also detected. On the whole, this study seems to support previous observations made from burial practices about a specific regional Neolithic pattern in the Paris Basin as well as bring new elements into discussion of social organisation in human populations.

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Introduction

Isotopic analyses have been conducted routinely since the 1970s to study the diet of past human populations. They provide new results to complement the data from other disciplines: studies on food remains (e.g. zooarchaeology, archaeobotany, lipid residue analysis on pottery, plant micro-remain studies on dental calculus), dental and skeletal health status studies (e.g. stress markers, caries, deficiencies, dental wear) and other archaeological evidence. While these studies generally concern the population's food regimes and are subject to the availability and the conservation of these remains, isotopic analyses bring complementary information allowing a direct and individual vision of the human diet. Some resources (meat, plants, fish) have distinct isotopic ratios that are recorded in the consumer's tissues. The analysis of these tissues allows each individual to be replaced in the local food chain and to access intra-group variations. In

human societies, such variations often depend on socio-cultural parameters. Analysis of local and contemporary animals both provides an isotopic baseline for human diet (as isotopic values vary with environmental conditions) and bring more indications on the livestock management by the human populations. The Neolithic period, in particular, is studied to document one of the biggest dietary transitions in human history: hunter-gatherer populations settle down and become farmer-pastoralists. New resources (animal and plant species newly domesticated) are introduced into the diet of human groups in varying proportions depending on several factors (resources availability, cultural choices, etc.). The Meso-Neolithic transition is well established through isotopic analyses and varies by region according to local specificities (e.g. Jovanović et al. 2018; Richards and Hedges 1999). Many sites in Europe have already been analysed for stable carbon (C) and nitrogen (N) isotope ratios in bone collagen and highlight the dietary changes that occurred in the Neolithic (e.g. Goude and Fontugne 2016). Sulphur (S) isotopic analyses are more rare but are increasingly being included in multi-proxy analyses to better document the dietary patterns, as well as the mobility patterns (e.g. Drucker et al. 2016b; Goude et al. 2018; Nehlich 2015).

In the Paris Basin, zooarchaeological data from Early and Middle Neolithic shows a consumption mainly of cattle (*Bos taurus*) followed by pig (*Sus scrofa domesticus*). Sheep (*Ovis aries*) and goats (*Capra hircus*) are in the minority (e.g. Bedault 2009; Hachem et al. 2014; Tresset 1989, 1997). Humans were also skilled in raising livestock, with dairy farming well-handled alongside meat production (Balasse et al. 1997; Balasse and Tresset 2002; Hachem et al. 2014). Wild animals (mainly deer – *Cervus elaphus*) were still hunted (Hachem et al. 2014) but rather for symbolic and cultural reasons, particularly during the Middle Neolithic (Sidéra 2003). Fishing should also be considered as an additional source of food even in a lesser extent, as a few fish remains have been found in some regional sites (Clavel and Arbogast 2007). Moreover, fishing is attested since the Mesolithic period in the region (Drucker et al. 2016b) and consumption of aquatic resources was proposed for a regional Neolithic site in a previous isotopic study (Rey et al. 2017). Besides, carpological and palynological regional data show that, in this temperate climate with C_3 vegetation, cereal cultivation is present since the early Neolithic with wheat (*Triticum dicoccum* and *monococcum*) and barley (*Hordeum vulgare*) (Bakels 1997; Martin et al. 2014). Wild species are also retrieved: peas (*Pisum sativum*), lentils (*Lens culinaris*), hazelnuts (*Corylus avellana*), wild herbs, etc. (Ilett et al. 1995; Martin et al. 2014). In the Yonne valley, the farming equipment found in several sites reflects the agricultural practices (grindstones, knobs and sickles have been found in Gurgy "Les Noisats" and "Les Grands Champs" and in Monéteau; Augereau and Chambon 2011; Meunier et al. 2006). In this context, isotopic analyses bring a new insight of the local human diet allowing comparisons with other contemporaneous

sites. Each isotope answers specific questions and the combination of several proxies provides a clearer view of the diet (Cooper et al. 2018). Studying bone collagen especially provides information about the proteins consumed, i.e. mainly about animal resources (as plants generally contain less proteins). Complementary studies better answer the issue of the plant part (e.g. archaeobotany and dental calculus analysis; Cristiani et al. 2018, Bouby and Léa 2006).

Furthermore, the Neolithic period is characterised by important cultural changes. The new way of life is spreading in Europe from the Near East through two routes of diffusion: one continental by the Danube, associated with the Linear Pottery Culture (LBK), the other in the south along the Mediterranean Sea associated with the Cardial culture. The Paris Basin is at the extreme point of the continental route. There is evidence the new economy was established from current Lorraine to Normandy shortly before the end of the 6th millennium BC (Billard et al. 2014; Bostyn 2003). In the south of the Paris Basin, the situation is a little different. The traces of this first continental Neolithic period are fleeting, while the centre of France also displays evidence related to the Mediterranean Early Neolithic. The interactions between the two spheres remain poorly understood, but they probably occurred as early as the first centuries of the 5th millennium BC in the south of the Paris Basin. The middle Yonne valley is potentially one of these contact areas (Chambon et al. 2013; Prestreau and Thevenot 1996). There, the LBK reached its southern margin and, from the beginning of the Middle Neolithic period, there is evidence of local cultural expressions that relate little to the rest of the Paris Basin. This area of contact is still marked by the juxtaposition, on a limited geographical scale, of distinct cultural entities (Rubané Récent du Bassin Parisien [RRBP], Blicquy-Villeneuve-Saint-Germain [BVSG], Cerny, Chasséen, Grossgartach, Roessen, Michelsberg). To date, little is known about their respective diet, particularly from the isotopic studies. These cultural entities are defined on the basis of archaeological characteristics and did not reveal specific isotopic signatures. However, some trends can be observed on a larger scale between the cultural spheres of the North and the South, due in particular to local environmental conditions (Goude and Fontugne 2016). However, it is difficult to compare different cultural entities when they do not belong to the same geographical or chronological unit.

The present study considers seven neighbouring sites, almost contemporaneous and attributed to different cultures. It is the biggest study (C, N, S) so far for the European Neolithic to be conducted on numerous human and animal[1] samples from a very limited area. The sites are located on the same geological formation (sedimentary layer c1—Early Cretaceous), along 10 km of the Yonne river in the Paris Basin (Fig. 1; Chambon et al. 2013). They are dated from the

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[1] For simplicity, in the text "animal" excludes humans.

Early to the Middle Neolithic. The radiocarbon dates of the human remains range between ca. 5500 and 3000 cal. BC. They concern 97 samples and are taken from the publications corresponding to each site cited in Table 1 (chronological distribution in Online Resource 1). The most recent dates are doubtful because they are too far away from the others for a coherent functioning of the cemetery: those after 3500 cal. BC are not considered in this study. The sample selection was made in such a way as to best represent the apparent cultural diversity. All the sites are funerary but of different types and size (large cemeteries or small complexes, enclosures, etc.) and are associated to several cultures. The grave goods and funerary practices mainly refer to the Cerny and the Chassean cultures, which are found rather in the northwest and southwest respectively, or represent a local component with a mixture of several surrounding influences (Fig. 1). Other archaeological and sampling information relative to these sites is summarised in Table 1 and detailed later.

Thus, the aim of this study is firstly to document the diet of the Neolithic populations of the Yonne valley compared to the adjacent regions. Are the isotopic signatures in the valley homogeneous? Is their position compared to the animals different from what is known elsewhere? What is the contribution of protein resources? Which livestock were mainly raised for food? Was fish possibly consumed? The second focus of this study concerns the inter- and intra-groups variability potentially correlated to socio-cultural status in order to discuss the social organisation and population identity. Are there specific patterns for the different sites, may be linked to their nature? For example, is the isotopic signature different between humans buried in large cemeteries and those scattered in enclosure ditches? Can distinctions related to cultural or funerary characteristics be identified, even within sites? Are there gender division or age disparities, as suggested in a previous study (Rey et al. 2017)? Are there outliers who could have a particular position in the society? The representativeness of the sample selection facilitates consistent comparisons with archaeo-anthropological data. An inclusive multi-proxy approach enables a thorough discussion of the subsistence strategies and the social structuring of human groups in a well-determined archaeological context like this one. Finally, these questions will provide new data to discuss the identity of the populations of the Yonne Valley, their economic and social functioning and, more broadly, their place in the European Neolithic landscape.

Materials and methods

Brief description of the sites

The seven sites considered in this study are rather diversified and need a brief description. They count three open-air cemeteries, two enclosures with scattered remains and 2 little ensembles with alignments of burials or levelled burial. They refer to different cultures and are dated to more or less later phases of the Early and Middle Neolithic period, although their chronologies overlap (see Online Resource 1 for the chronological distribution of the sites). The main information is summarised in Table 1 and complementary information with plans of the sites is presented in Online Resource 2.

Gurgy "Les Noisats" (coded GLN) is the biggest corpus of this study and one of the largest cemeteries (in terms of number of burials) for the French Neolithic. It contains more than 120 burials (Online Resource 2a) dated to 5200–3800 cal BC and the great diversity of the grave goods (although few in number) and of the funerary structures refer to several surrounding cultures (Augereau unpublished; Bonnardin unpublished; Rivollat et al. 2015; Rottier 2007; Rottier et al. 2005). That makes it impossible to attribute the cemetery to one of these cultures specifically. Several studies (physical anthropology, genetic, dental morphology and previous isotopic analyses) have shown a rather homogeneous population (all the classes share the same characteristics) but some slight variations have been highlighted between individuals: disparities between genders, clusters of nearby graves sharing similar characteristics and some outliers displaying unusual funerary parameters (notably GLN 221B who occupies a central place in a very large tomb). The previous C and N isotopic results are included into the present study. The second largest site is that of Monéteau "Macherin" (coded MON). It contains the second biggest cemetery of this region, with 61 burials at least partly attributed to the Chasséen culture (Augereau and Chambon 2011). It also includes a settlement with an associated burial dated to the Early Neolithic and an enclosure, without human remains, contemporaneous to the cemetery. The latter is divided in three groups of burials (A and B inside the enclosure, C outside) and two isolated pairs of burials (D and E; Online Resource 2b). Group C, the biggest one, is contemporaneous to the main occupation of Gurgy "Les Noisats" (mainly 5000–4200 cal BC) and similarly dense in burials. Groups A and B may be more recent (4500–4000 cal BC) and narrower. No other characteristics distinguish the humans buried in these groups. Generally, the funerary behaviours are rather homogeneous, with many coffins and bodies buried flexed on the left side, but some burials can also be associated with other cultural affiliations (e.g. bodies extended supine as Balloy-type, like in the Cerny culture). Chichery "Sur les Pâtureaux" (mainly 4700–4350 cal BC; coded CHI-Pat) constitutes the third main site of the corpus. It is attributed to the Cerny culture (Chambon et al. 2010) but, contrary to the most cases in this culture, it is not considered as a monumental structure (Thomas 2011). Some funerary parameters also refer to other cultures (Chambon et al. 2010). Thirteen burials containing 15 humans are arranged in two main alignments (A and B) where individuals are extended on the back, according to Balloy's type
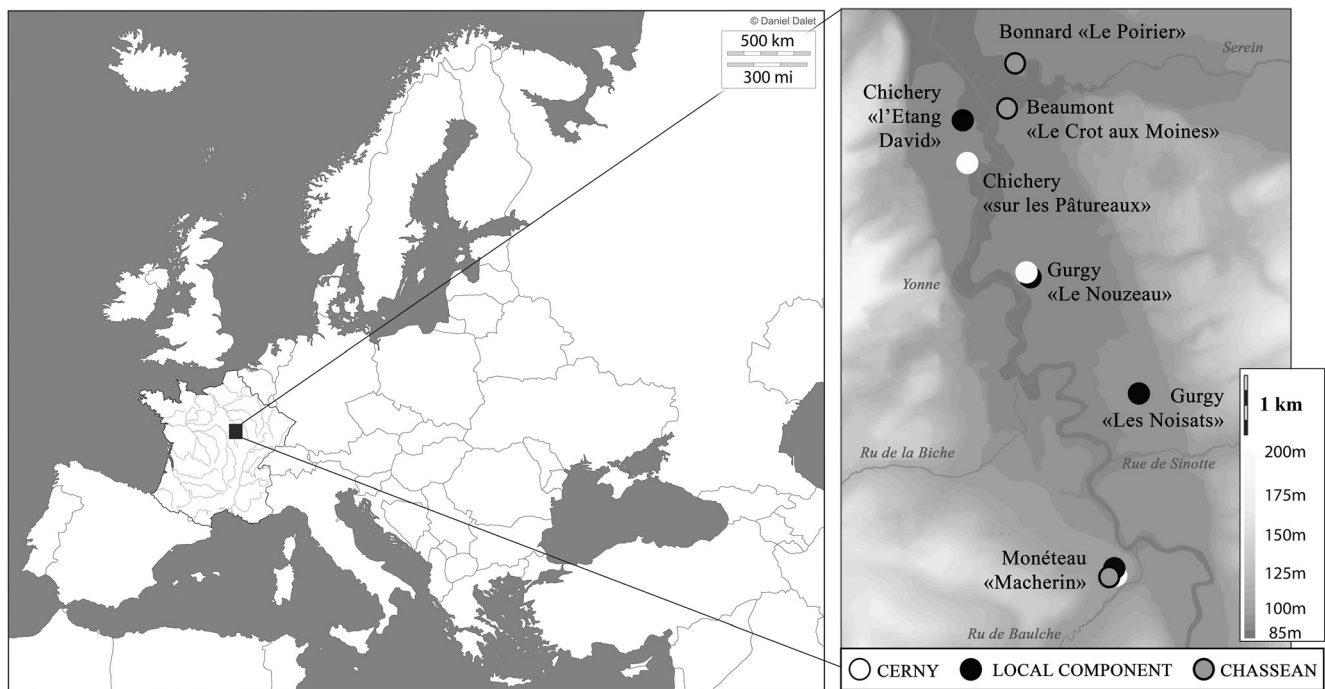
Fig. 1 Locations of the sites analysed in this study, and their respective cultural attributions, after Chambon et al. 2013

(Chambon et al. 2009b; Online Resource 2c). One isolated burial contains the body of a man flexed on his left side and dated to a later period (4335–4074 cal BC).

The following sites are not used for comparing isotopic with biological data of humans (age and sex) because they contain too few remains for which these parameters are known. Beaumont "Le Crôt aux Moines" (4800–4000 cal BC; coded BMT; Online Resource 2d) is an enclosure attributed to Chasséen containing scattered remains of humans and animals together (Chambon et al. 2009a). Other structures were also retrieved, from several excavations, so the site counts 3 main areas: Area A (Pellet and Delor excavation) with the circular house, the fireplace 2 and the s13 78 A II gh area; Area B (Merlange and Bodechon excavation) with the enclosure consisting in two ditches (A and B) that contain the most of the material; and the North area (surface sampling) with the layer 5 (Ederlé prospection) and layer IV. Bonnard "Le Poirier" (4500–4000 cal BC; coded BON) is a little Chasséen ensemble (anciently and partially excavated) (Chambon 2003) composed of an isolated levelled burial with 4 adult individuals and another pit with human remains from 2 individuals severely disturbed (Online Resource 2e). Chichery "l'Etang David" (5100–4100 cal BC; coded CHI-ED) is an incomplete cemetery, partially destroyed and excavated a long time ago (Chambon et al. 2009a; Pellet 1978). It displays 19 burials from which only 5 skeletons have been retrieved (Online Resource 2f). Gurgy "Le Nouzeau" (5300–4700 cal BC; coded NZ) is a Cerny enclosure composed of human and animal scattered remains in the ditch and 2 associated burials of adult men (Meunier 2007; Meunier et al. 2012; Online Resource 2 g).

Only the latter were sampled for this study together with animals from the ditch. These two men were buried in exactly the same position and in quite similar graves. However, they are dated to different ^{14}C ranges, which suppose some dating error.

Human and animal bone sampling

Overall, stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope analyses were performed on 177 humans and 62 animals, and stable sulphur isotope analysis ($\delta^{34}S$) was conducted on 52 humans and 25 animals (Table 1; Online Resource 3). Bone samples have been selected so that they encompass as much as possible the archaeological and anthropological diversity observed: different sites, spatial distribution, chronology, funerary parameters—structures, positions, grave good types—and sex, age and genetic data, when available. Long bone cortex (especially from the femur and tibia) was preferentially chosen. The biological identification of humans (sex, age-at-death) was previously studied with reliable methods (Bruzek 2002; Moorrees et al. 1963; Murail et al. 2005). Immature individuals under 2 years old were excluded to avoid, where possible, the breastfeeding signal (limit defined considering the class-age division; the suckling signal has not been overlooked in older children). Moreover, M. Le Roy (2015, p. 440–442) noted changes in funerary behaviours around 14 years of age, mainly at GLN, reflecting a change of social status. Considering this, together with the class-age division, individuals over 15 are considered in this study as part of the adult group. Animal remains come from three sites (BMT, GLN and NZ) and include domestic (cattle, pig, sheep/goat)

Table 1 Summary of the sites' description and references, 14C dates, and human and animal sample selection.

code site	name of site *old excavation	references	cultural attribution	14C date	spatial organization	number of humans buried	animal remains	δ13C & δ15N individuals analysed	δ34S individuals analysed
GLN	Gurgy "Les Noisats"	Rottier et al 2005, Rottier 2007, Rivollat et al 2015	local component ?	5200-3800 cal BC	big and dense cemetery; some gathering of burials; great diversity of grave goods	128	yes	95 humans + 18 animals	28 humans + 9 animals
MON	Monéteau "Sur Macherin"	Augereau & Chambon 2011	Chassean (+Cerny +local)	5200-3500 cal BC ?	cemetery divided in 3 separate groups (A, B, C) + 2 pairs of burials (D, E) + 1 earlier burial in settlement	62	no	53 humans	18 humans
CHI-Pat	Chichery "Sur les Pâtureaux"	Chambon et al 2010	Cerny	4700-4350 cal BC	funerary site arranged into 2 aligments of burials (A and B; Balloy's type) + 1 isolated burial (male flexed on his left side)	15	no	8 humans	6 humans
BMT	Beaumont "Le Crôt aux Moines" *	Chambon et al 2013	Chassean	4800-4000 cal BC	enclosure + other structures; 3 areas of excavation. Area A: circular house ($\approx$ 4300-4000 cal BC), fireplace 2 ($\approx$ 4400-4100 cal BC), and area s13 78 A II gh ($\approx$ 4700-4400 cal BC). Area B (Merlange-Bodechon excavation): enclosure with scattered remains in 2 ditches A and B ($\approx$ 4800-4200 cal BC); the best-documented area and having provided the most of material. North area: surface sampling layer 5-Ederlé ($\approx$ 4500-4300 cal BC) and layer IV.	scattered remains	yes	13 humans + 38 animals	13 animals
BON	Bonnard "Le Poirier" *	Chambon et al 2013	Chassean	4500-4000 cal BC	1 levelled burial of 4 inhumations + 1 pit with 2 individuals severely disturbed	6	no	4 humans	
CHI-ED	Chichery "Etang David" *	Pellet 1978, Chambon et al 2013	local component ?	5100-4100 cal BC	cemetery partially destroyed	$\approx$ 20 (5 skeletons retrieved)	no	2 humans	
NZ	Gurgy "Le Nouzeau"	Meunier et al 2012	Cerny (+ local)	5300-4700 cal BC	enclosure with scattered remains (humans and animals) in ditches + 2 associated burials	2 + scattered remains	yes	2 humans + 6 animals	3 animals

and wild (deer—*Cervus elaphus* and *Capreolus capreolus*, aurochs—*Bos primigenius*) species (Online Resource 3). Sampling was carried out in such a way that we ensured that every individual was only sampled once (selection by species, anatomical part, size and shape, spatial location).

Principle of stable isotope analysis

At the bottom of the food chain, plants have specific C, N and S isotopic ratios according to their photosynthetic pathway (e.g. C_3 or C_4; Farquhar et al. 1989; O'Leary 1988; Smith and Epstein 1971), their species (e.g. N_2 fixing or non-N_2 fixing plants; DeNiro and Epstein 1981; Virginia and Delwiche 1982), and their environment (Ambrose 1991; Hedges et al. 2004; Van Klinken et al. 2000; Richards et al. 2003; Tanz and Schmidt 2010). These values are then passed through the food chain and register in the consumer's bone collagen with an enrichment between prey and predator of 0.8 to 1.3‰ for $\delta^{13}C$, 3 to 5 ‰ for $\delta^{15}N$, and negligible enrichment for $\delta^{34}S$ (Bocherens and Drucker 2003; DeNiro and Epstein 1978, 1981; Minagawa and Wada 1984; Schoeninger and DeNiro 1984; Arneson and MacAvoy 2005; Barnes and Jennings 2007; McCutchan et al. 2003). Individuals' place in the local food chain can be retrieved by measuring these ratios in bone collagen, thus determining their trophic level, their potential consumption of the different species and providing environmental information. Because the environmental conditions (temperature, humidity, edaphic properties, etc.) can influence the isotopic values, human data must be compared to those of local and contemporary animals which could potentially have been consumed (Goude and Fontugne 2016; Heaton 1999; Heaton et al. 1986; Hedges et al. 2004; Van Klinken et al. 2000). Plants' $\delta^{34}S$ values are particularly variable according to environment and geography because they are affected by the local bedrock as well as atmospheric and edaphic conditions. Therefore, measuring the $\delta^{34}S$ of the consumers can allow to identify distinct origins (e.g. Richards et al. 2001) and/or, depending on locality, also distinguish various animal species consumed (e.g. Privat et al. 2007). It can be particularly useful to detect freshwater fish consumption (Drucker et al. 2016b) and, as this was proposed in a previous preliminary study conducted on C and N isotope ratios in the Yonne valley (Rey et al. 2017), this is the main reason for measuring it in this study. Despite increasingly more sulphur data is now being made available in various contexts, geological and bioavailable sulphur variation at local or sublocal scales is still poorly known (e.g. Nehlich 2015). Moreover, the C, N and S multi-proxy approach is quite new for the Neolithic in France and seems very helpful to enhance our knowledge of the human diet and corresponding behaviours. Furthermore, the isotopic composition

measured in bone collagen mainly reflects the protein portion of the average diet recorded during the last years of life (several decades for adults). Indeed, bone is constantly being remodelled, with a highly variable rate depending on several factors, such as type of skeletal element and bone part, age, growth hormones, disease and nutritional status (Hedges et al. 2007; Valentin 2003; Tieszen et al. 1983; Szulc et al. 2000; Delmas 1993).

Analytical process

Bone fragments (< 1 g) were cleaned and crushed, and collagen was extracted (UMR 7269 LAMPEA, France) following Longin's (1971) and Bocherens' methods Bocherens (1992). Elemental composition (%C, %N, %S) and isotopic ratios ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$) were measured on freeze-dried collagen by EA-IRMS (elemental analyser–isotope ratio mass spectrometer). Part of the measurements was performed using a Europa scientific elemental analyser coupled to 20–20 IRMS at the IsoAnalytical laboratory (UK). The other part was performed using a Delta plus XP continuous-flow IRMS (Thermo Fisher Scientific, USA), after being combusted in a Flash EA 1112 elemental analyser that was interfaced with it (Thermo-Finnigan, USA), in the Isotope Lab Facilities of the University of Cape Town (South Africa; see Online Resource 3). Sample isotopic values are reported relative to the international reference scales V-PDB for C, AIR for N and V-CDT for S isotopes. The reference materials used for calibrating the isotopic data were internal standards calibrated against IAEA standards (Table 2). Reproducibility error, based on within-run replicate measurement of laboratory standards was ± 0.1 ‰ for $\delta^{13}C$ and $\delta^{15}N$, and ± 0.2 ‰ for $\delta^{34}S$. The quality of extracted collagen was controlled by using international criteria: collagen yield > 1%, C > 30%, N > 11%, C:N ratio of 2.9–3.6 (Ambrose 1990; DeNiro 1985; van Klinken 1999), S content of 0.15–0.35%, C:S ratio of 600 ± 300, and N:S ratio of 200 ± 100 (Nehlich and Richards 2009). For statistical processing, all the analyses were conducted with the software R 3:5.2 (R Core Team 2018). The code and the source data are available in Online Resource 4 and 5. Given the presence of a few outliers, non-parametric tests were applied to compare isotopic values among groups. Post hoc comparisons for the Kruskal-Wallis test were performed using Holm's method for adjusting p values, as implemented in the R package "agricolae" (de Mendiburu 2017). Multivariate isotopic profiles were compared among groups using M.J. Anderson's (2001) variant of permutational multivariate analysis of variance (PERMANOVA) with 1000 permutations, as implemented in the R package "vegan" (Oksanen et al. 2017).

Table 2 Summary of the reference materials used for calibrating the isotopic data in the different laboratory and by session of analyses. Internal standards are calibrated against international standards.

lab	reference	internal standard	material	N measured	standard deviations			accepted values			international standard	material	accepted values		
					$\delta^{13}C_{V\text{-}PDB}$ (‰)	$\delta^{15}N_{AIR}$ (‰)	$\delta^{34}S_{V\text{-}CDT}$ (‰)	$\delta^{13}C_{V\text{-}PDB}$ (‰)	$\delta^{15}N_{AIR}$ (‰)	$\delta^{34}S_{V\text{-}CDT}$ (‰)			$\delta^{13}C_{V\text{-}PDB}$ (‰)	$\delta^{15}N_{AIR}$ (‰)	$\delta^{34}S_{V\text{-}CDT}$ (‰)
IsoA	Rey et al, 2017	IA-R042	NBS-1577B, bovine liver	10	0.06	0.07		-21.60	7.65		IAEA-CH-6	sucrose	-10.43		
		IA-R005 / IA-R045	beet sugar / ammonium sulfate	5	0.01	0.04		-26.03	-4.71		IAEA-N-1	ammonium sulfate		0.40	
		IA-R006 / IA-R046	cane sugar / ammonium sulfate	5	0.09	0.08		-11.64	22.04						
IsoA	this study	IA-R042	NBS-1577B, bovine liver	3	0.11	0.12		-21.60	7.65		IAEA-CH-6	sucrose	-10.43		
		IA-R038	L-alanine	2	0.00	0.02		-24.99	-0.65		IAEA-N-1	ammonium sulfate		0.40	
		IA-R006 / IA-R046	cane sugar / ammonium sulfate	1				-11.64	22.04		IAEA-SO-5	(barium sulfate)			0.50
		IA-R001	barium sulfate	28			0.10			20.33					
		IAEA-SO-5	barium sulfate	15			0.16			0.50					
		NBS-1577B	bovine liver	15			0.24			7.50					
UCT	this study	Merck Gel		19	0.13	0.07		-20.05	7.50		IAEA-CH-6	sucrose	-10.43		
		Seal		19	0.13	0.05		-11.97	15.84		IAEA-N-1	ammonium sulfate		0.40	
		Valine		19	0.12	0.07		-26.80	12.14						

Results and comparison

General results

All the individual results are provided in Online Resource 3. Twenty-eight samples (22 humans[2] and 6 animals) did not meet the collagen preservation criteria for $\delta^{13}C$ and $\delta^{15}N$, and only one sample (human) did not meet the preservation criteria for $\delta^{34}S$ collagen (Online Resource 3). These have been excluded from the study. In total, 211 samples for $\delta^{13}C$ and $\delta^{15}N$, and 76 samples for $\delta^{34}S$ have been included in the interpretation and discussion. The isotopic ratios do not correlate to elemental composition and extraction yield (Spearman correlation coef. < 0.2). Therefore, the range of values does not reflect diagenetic alterations.

The overall C and N isotope ratios have been plotted onto Fig. 2a and are summarised by species in Table 3. Three different groups of data can be clearly identified, representing different feeding behaviours: humans, pigs and domestic herbivores. The latter is composed of sheep and goats (i.e. Caprinae) together with cattle: they present broadly the same range of values. The difference between these three groups is statistically significant when considering the bivariate ($\delta^{13}C$, $\delta^{15}N$) isotopic profile according to PERMANOVA ($p < 0.05$). Wild animals show lower $\delta^{13}C$ and $\delta^{15}N$ values than domestic ones, but the difference is not significant.[3] The distribution of the human $\delta^{13}C$ values is very low (SD $\delta^{13}C = 0.2‰$) and is greater for the animal species (SD $\delta^{13}C$ herbivores = 0.8, pig = 0.7 and wild = 0.9‰). These differences in variability between humans and all animal groups are statistically significant according to Snedecor's F test ($p < 0.05$).

The S isotopic data are plotted against $\delta^{15}N$ (Fig. 2b) and shows four separate groups: humans, domestic herbivores, pigs (this category is also divided into two sub-groups, from GLN and BMT respectively), and wild animals (Table 3). Excluding two outliers (one cattle, GLN F0, maybe wild and one pig BMT F5 out of the overall variability), a Kruskal-Wallis test detected significant differences in $\delta^{34}S$ values among the groups compared ($p < 10e-5$). Furthermore, a post hoc analysis shows that the differences between cattle, humans and wild animals are all statistically significant. Here, cattle are considered separately from Caprinae because the dispersion of their $\delta^{34}S$ values is more dissimilar than the dispersion of the C and N isotopic data. Additionally, pig $\delta^{34}S$ values are clearly divided into two groups and the difference is statistically significant (test as above). The GLN pigs' group is similar to the humans, the BMT one to the wild animals (test

as above). Overall, the S isotopic variability is very huge (from − 10 to + 15.1‰), particularly given all samples came from a very small area.

Animals by sites and areas

The isotopic distribution of animal values for each site ($n = 56$ for C and N and 25 for S) is presented in Figs. 3 and 4 and mean values by species are available in Table 4. For each site, the stable isotope data (C, N and S) of pigs is higher than those of cattle. This result is significant for C and N in both GLN and BMT (Mann-Whitney U test, $p < 0.05$). Cattle and Caprinae share similar C, N and S values at BMT but have different C, N and S values at GLN and NZ (although the sample size is small). At GLN and NZ, wild species show different isotopic values (lower $\delta^{13}C$ and $\delta^{15}N$, and higher $\delta^{34}S$) than domestic ones (reliable statistical analysis cannot be performed due to the small sample size). This difference (domestic vs. wild) is not as clear at BMT, but this site is divided into several separated areas and structures that must be considered separately. Indeed, animal C, N and S isotopic values of BMT vary slightly between areas (see Online Resource 6 for more details by area). Particularly, the deer with the highest N isotopic ratio and the cattle with the lowest N and C isotopic ratios come from layer 5 "Ederlé" and cannot be attributed with certainty to any specific phase of occupation (surface collection). Excluding these samples, the picture between wild and domestic animals is nearly the same than in the other sites (Fig. 3a). Furthermore, pigs found in the circular house exhibit substantially different isotopic ratios (C, N and S) than in other areas (Online Resource 6), although the small sample sizes do not allow to perform statistical tests. At GLN, domestic animals show slightly different isotopic ratios to those from BMT and NZ. In this site, cattle (like all the domestic animal groups taken together) have a higher $\delta^{15}N$ value (Mann-Whitney U test, $p < 0.05$) and pigs have higher $\delta^{13}C$ and lower $\delta^{34}S$ values (Mann-Whitney U test, $p < 0.05$, excluding the outlier for S) compared to BMT. The comparison with NZ is less relevant due to the little number of samples.

Humans by sites and areas

Regarding the human variability, 155 humans from the seven sites have provided well-preserved collagen and reliable isotopic data for carbon and nitrogen, and 51 from three sites for sulphur (Fig. 5 and Table 5). The isotopic data show some distinctions between sites for nitrogen and sulphur whereas carbon isotopic values are similar. The human group from GLN presents the highest $\delta^{15}N$ and lowest $\delta^{34}S$ values, while the contrary (highest $\delta^{34}S$ and lowest $\delta^{15}N$ values) is observed among the MON people. Post hoc comparisons show that for

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[2] NZ 239 has been accepted though his extraction yield was insufficient; all the other criteria were acceptable.
[3] Some specimens were not identified as domestic with certainty, but have a high probability of being domestic specimens and are consequently considered as such; cf Online Resource 3 noted *
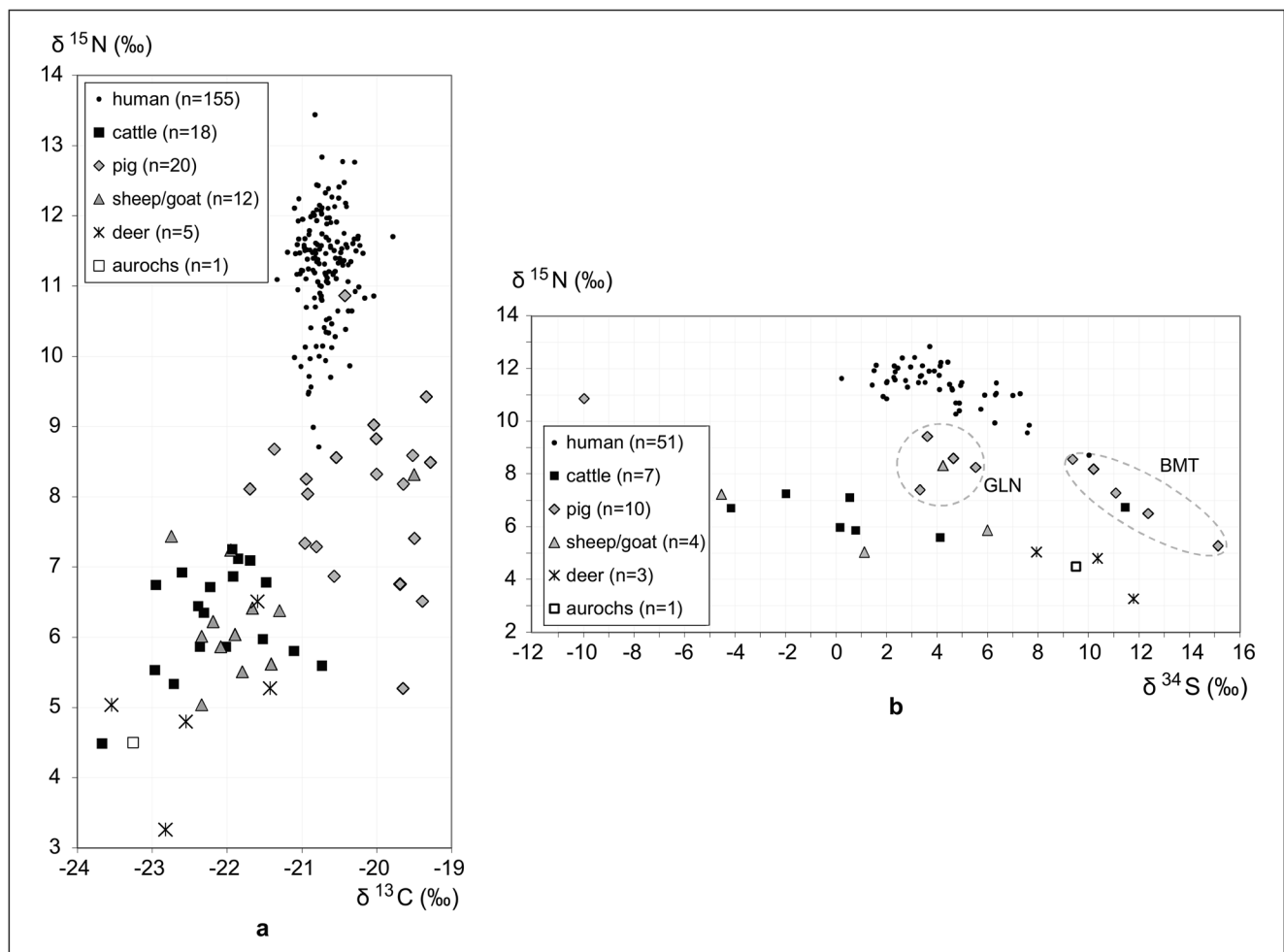
Fig. 2 Distribution of isotopic values for all the samples retained, all sites included **a** 155 humans and 56 animals for carbon and nitrogen and **b** 51 humans and 25 animals for sulphur and nitrogen. The dotted circles show the separate distributions of pigs from GLN and BMT

δ^{15}N, GLN statistically differs (higher) from a homogeneous ensemble constituted by CHI-Pat/MON/BMT and, for δ^{34}S, GLN statistically differs (lower) from MON only. The variation of human values at GLN is relatively limited and significantly lower than the one observed at MON, for both δ^{15}N ($p = 0.02$) and δ^{34}S ($p = 0.02$) values according to Snedecor's F test. The high variability observed at MON seems to reflect the spatio-temporal structuring of the site. Indeed, MON is divided in three separate groups (A, B and C), two isolated pairs of burials (D and E) and one earlier isolated burial (Online Resource 2b). The different groups display different ranges of isotopic values for C, N and S (Figs. 6b and 7b). The difference between group B, group C and the other groups together is statistically significant for δ^{15}N (Kruskal-Wallis test, $p < 0.05$). Group C variation is closer to the GLN one, while both complexes share similar archaeological patterns and are somewhat contemporary. Group B displays the lowest δ^{15}N and the highest δ^{34}S values of the overall corpus and is also among the most recent burial assemblages. This correlation with

chronology is the only one observed in this study that reveals a change of isotopic values (as an average, not in variability) through time between sites or areas (see Online Resource 7 for more details).

The individuals from CHI-Pat present relatively homogeneous and medium C, N and S isotopic values compared to the overall human variability (Fig. 5). The humans of this site have lower δ^{15}N values than humans from GLN (Mann-Whitney U test, $p < 0.05$) and these values have a significantly lower dispersion than for other human groups according to Bartlett's test ($p < 0.05$). Despite this relatively low variability at the site level, the two groups of CHI-Pat (consisting of two alignments of burials) have distinct values, especially for nitrogen and sulphur (Figs. 6c and 7c; sample size too small for statistical analysis). The isolated individual CHI-Pat 1, buried in another position than the others (flexed on his left side), has the highest C and S isotope ratios of the site (CHI-Pat) although these values are not very different from the other humans. Individuals from BON and NZ display similar, low nitrogen isotopic values compared to the overall human data

Table 3 Summary isotopic results (δ13C, δ15N, and δ34S) by species

Species	N	$\delta^{13}C$ (‰)				$\delta^{15}N$ (‰)				N	$\delta^{34}S$ (‰)			
		min	**mean**	*SD*	max	min	**mean**	*SD*	max		min	**mean**	*SD*	max
TOTAL human	155	-21.3	**-20.7**	*0.2*	-19.8	8.7	**11.3**	*0.8*	13.4	51	0.2	**4.1**	*1.9*	10.0
TOTAL pig	20	-21.7	**-20.2**	*0.7*	-19.3	5.3	**8.0**	*1.2*	11.0	10	-10.0	**6.5**	*7.0*	15.1
cattle	18	-23.7	**-22.1**	*0.7*	-20.7	4.5	**6.3**	*0.7*	7.3	7	-4.2	**1.6**	*5.1*	11.4
sheep/goat	12	-22.7	**-21.8**	*0.8*	-19.5	5.0	**6.3**	*0.9*	8.3	4	-4.5	**1.7**	*4.6*	6.0
TOTAL herbiv	30	-23.7	**-22.0**	*0.8*	-19.5	4.5	**6.3**	*0.8*	8.3	11	-4.5	**1.6**	*4.7*	11.4
deer	5	-23.5	**-22.4**	*0.9*	-21.4	3.3	**5.0**	*1.2*	6.5	3	7.9	**10.0**	*1.9*	11.8
aurochs	1	-23.3	**-23.3**		-23.3	4.5	**4.5**		4.5	1	9.5	**9.5**		9.5
TOTAL wild	6	-23.5	**-22.5**	*0.9*	-21.4	3.3	**4.9**	*1.1*	6.5	4	7.9	**9.9**	*1.6*	11.8

(Fig. 5) although they are dated to different periods. The difference with GLN is statistically significant for $\delta^{15}N$ according to a Mann-Whitney U test ($p < 0.05$). The three individuals from BON have very similar C and N isotopic values (Fig. 5). Conversely, the two people buried at Chi-ED show very distinct values, especially for nitrogen (Fig. 5). Finally, humans from BMT have the same $\delta^{13}C$ and $\delta^{15}N$ mean values than CHI-Pat but a wider $\delta^{15}N$ range and a higher variance (Bartlett's test, $p < 0.05$). In BMT, there is no clear distinction between the different areas (Online Resource 6C) although some differences were observed within the animal samples. Considering the distribution of the isotopic ratios by sites, it appears that there is no correlation between isotopic data and nature of the sites. The isotopic results of the enclosures of NZ and BMT are no more close to one another than those of the cemeteries of GLN, MON or CHI-ED are. There is no more link with the main cultural attribution of the sites. NZ and BON, attributed to different cultures and periods, have similar isotopic ratios. When considered as an entity, CHI-Pat and NZ, attributed to the Cerny culture, are not distinct from GLN and CHI-ED when these two sites, which share some cultural patterns characteristic of the Yonne valley and are dated to the same period, are also considered as a unit. There are no more similarities between BON and BMT, both attributed to the Chasséen, than with the other sites attributed to other cultures.
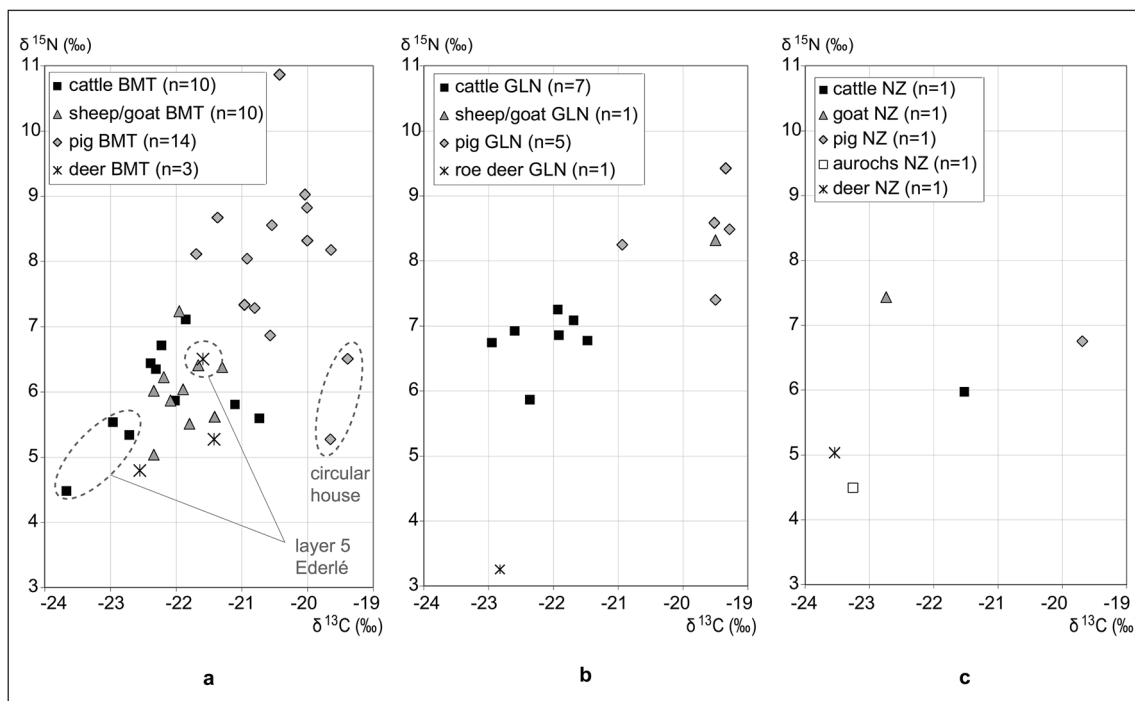

Fig. 3 Distribution of animal $\delta^{13}C$ and $\delta^{15}N$ values from **a** BMT, **b** GLN and **c** NZ. The dotted circles show the particular values of species from different areas of BMT
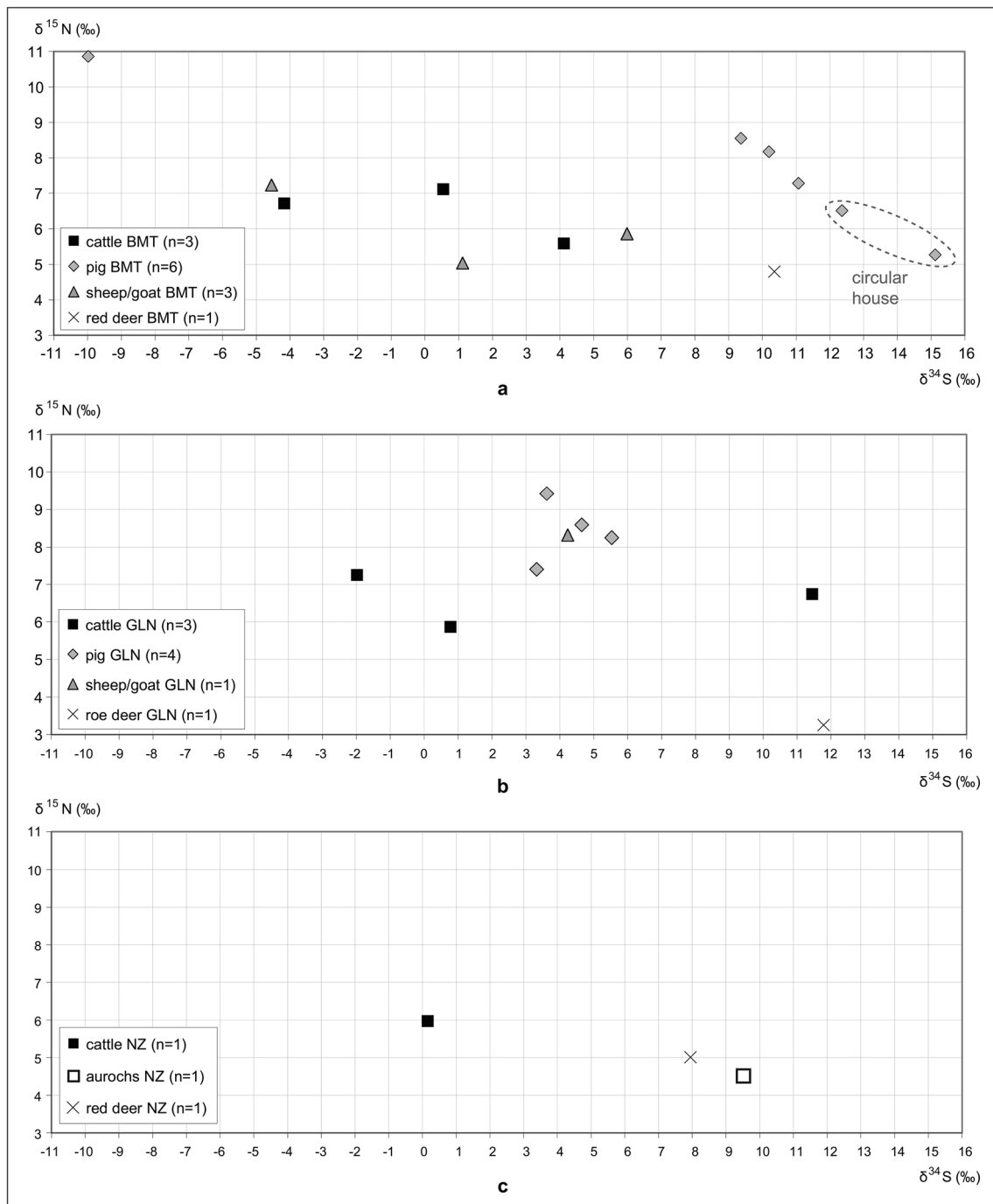
Fig. 4 Distribution of animal δ^{34}S and δ^{15}N values from **a** BMT, **b** GLN and **c** NZ. The dotted circle shows the particular values of pigs from the circular house of BMT

Intra-group isotopic variability of humans

In three sites for which biological data are available and are consistent enough for statistical analysis (GLN, MON, CHI-Pat), it was possible to combine isotopic data with age and sex (Figs. 6 and 7). For each site and within groups, males show different isotopic values (higher δ^{13}C and δ^{15}N, and lower δ^{34}S, on average) than females, as it had already been observed in a previous study for GLN (Rey et al. 2017). The difference is statistically significant for GLN and for MON group C for δ^{13}C and δ^{15}N (Wilcoxon test, $p < 0.05$; sample size too small in the other groups for statistical analysis). For each group, the highest δ^{15}N values of adults are recorded among the males and the lowest are recorded among the females. No significant

difference is detected in terms of variation of values between males and females. Children often display extreme values, particularly for nitrogen. The variability of children's $\delta^{15}N$ values is higher than those for adults (Bartlett's test, $p < 0.05$). However, no correlation was found between isotopic ratios and age, neither for children nor for adults.

Other comparisons with the available biological parameters (haplogroups, skeletal and dental health, or stature, information which is often sparse) do not correlate with the isotopic data and will not be further discussed. Regarding the burial practices, there is no general correlation (by site and by group, or between groups) between funerary parameters (type of burial, position and orientation of the bodies, grave goods) and isotopic data, despite the great variety of burials. However, some spatial distinctions can be observed. While MON, CHI-Pat and BMT are considered as several groups separately and discussed above (3.3), GLN seems to be a single ensemble. However, in this site, individuals buried near one another in the cemetery often have relatively close isotopic values compared to the overall variation (Fig. 8). Moreover, a group of individuals (GLN 201-202-206-207A-208-248) buried in an alcove or in a wettling pit, and gathered together at the south-western end of the cemetery, presents similar $\delta^{15}N$ and/or $\delta^{13}C$ values (light blue dots in Fig. 8; GLN 206 and 208 under 7 years of age). In addition, it is worth noticing that the individual previously identified as outlier GLN 221B (biggest and central tomb) presents the highest $\delta^{15}N$ value. In that site, the comparison between isotopic data and chronology does not show any change of isotopic values through time, but seems to show a variation in dispersion of the isotopic values (Fig. 9). The dispersion of $\delta^{15}N$ values is slightly higher during the period from 4800 to 4500 cal. BC, although this period has the most individuals and therefore the variability maybe due to a sampling bias. Furthermore, during this period, the $\delta^{15}N$ values seem to be more variable for men than for women (Fig. 9b).

Discussions

Food economy

First of all, the C, N and S isotopic differences highlighted between the species (domestic herbivores, pigs and wild animals) are quite similar for the three sites. They can be due to specificities inherent to the species themselves (relative to their respective diet, environment and physiology) and/or to specialised managements (different feeding or coming from different location). Namely, wild species probably live in a more covered environment than domestic animals (as forest, with a more negative $\delta^{13}C$ value; Heaton 1999); cattle, sheep and goats are herbivorous and may be parked and fed in open-air pens, separately from the pigs ($\delta^{34}S$ values are particularly

different from those of pigs and wild animals); pigs are omnivorous ($\delta^{15}N$ values are higher than for the herbivorous) and may be fed in part with human wastes (with a global diet richer in ^{13}C than the human diet). Such a distribution is not always observed elsewhere (Fig. 10, Online Resource 8). Concerning the variations, for a same species, between sites and areas, they could be due to chronological reasons or cultural/practical choices in livestock management. Since they concern different chronological phases between sites and areas, it is possible these variations indicate a change of environment through time, affecting differently the various isotopic ratios and the species. Also, the livestock could be kept in different spaces (e.g. enclosed with fodder vs. left roaming free to feed, more or less open canopy; Heaton 1999) and/or fed with different fodder (see Bogaard et al. 2007; Kanstrup et al. 2012). Such a diversified exploitation has been proposed in other studies (e.g. Oelze et al. 2011). The particularly high range of $\delta^{34}S$ values, while the sites are within a radius of 10 km and in the same geological area along the Yonne valley, can also illustrate different origins of the animals, especially for pigs from GLN vs. BMT areas. Also, despite their small number, wild animals which are considered as non-migratory appear to have a tighter range (from + 7.9 to + 11.8‰, $n = 4$) than the domestic ones (from − 10.0 to + 15.1‰, $n = 21$). That could illustrate a specific husbandry practice with livestock coming from elsewhere. This is particularly evident when comparing with, for example, the variation measured at Noyen-sur-Seine, a nearby Mesolithic site, for non-carnivorous terrestrial wild animals (from + 0.2 to + 4.7 ‰, $n = 9$, Drucker et al. 2016b). For pigs, another possibility involves species identification: the wild or domestic status of pigs has not been determined with certainty. Wild boars can display lower $\delta^{13}C$ and $\delta^{15}N$ values than domestic pigs (e.g. Balasse et al. 1997; Bocherens et al. 2005; Fig. 10, Online Resource 8). As the pigs of BMT have lower $\delta^{13}C$ and $\delta^{15}N$ values than the pigs of GLN, it is therefore possible that the bone samples belong to wild species instead of domestic ones (currently, a more in-depth study of the teeth is ongoing) or that domestic pigs from BMT eat the same thing as wild boars (thus running at large). This may be consistent with the sulphur values, as the pigs of BMT clearly plot with the wild species. Anyway, despite some variations between sites and particularly for pigs, the specific isotopic signatures of each species can be used as a baseline for interpreting the human diet.

The overall comparison between humans and domestic animals shows a mixed consumption of herbivores (probably mainly cattle according to the zooarchaeological data) and pigs. No distinction between meat and dairy consumption can be made through isotopic studies in bone collagen, but regional studies highlight a mixed production (Balasse et al. 1997; Hachem et al. 2014). While a mixture of cattle, pig, and caprine meat consumption is easily supposed for humans according to isotopic and zooarchaeological data, assessing the proportions of these various species in the diet is more

Table 4 Summary isotopic results (δ13C, δ15N, and δ34S) of animal samples by species for each site. TOT = total, herbiv = herbivorous, dom = domestic animals

animals

	animal species	N	δ13C (‰)				δ15N (‰)				N	δ34S (‰)			
			min	mean	SD	max	min	mean	SD	max		min	mean	SD	max
GLN (14)	cattle	7	-23.0	**-22.1**	0.5	-21.5	5.9	**6.8**	0.4	7.3	3	-2.0	**3.4**	7.1	11.4
	sheep/goat	1	-19.5	**-19.5**		-19.5	8.3	**8.3**		8.3	1	4.2	**4.2**		4.2
	TOT herbiv	8	-23.0	**-21.8**	1.1	-19.5	5.9	**7.0**	0.7	8.3	4	-2.0	**3.6**	5.8	11.4
	pigs	5	-20.9	**-19.7**	0.7	-19.3	7.4	**8.4**	0.7	9.4	4	3.3	**4.3**	1.0	5.5
	TOT dom	13	-23.0	**-21.0**	1.4	-19.3	5.9	**7.5**	1.0	9.4	8	-2.0	**3.9**	3.9	11.4
	deer	1	-22.8	**-22.8**		-22.8	3.3	**3.3**		3.3	1	11.8	**11.8**		11.8
BMT (37)	cattle	10	-23.7	**-22.2**	0.9	-20.7	4.5	**5.9**	0.8	7.1	3	-4.2	**0.1**	4.2	4.1
	sheep/goat	10	-22.3	**-21.9**	0.4	-21.3	5.0	**6.0**	0.6	7.2	3	-4.5	**0.9**	5.3	6.0
	TOT herbiv	20	-23.7	**-22.1**	0.7	-20.7	4.5	**6.0**	0.7	7.2	6	-4.5	**0.5**	4.3	6.0
	pigs	14	-21.7	**-20.4**	0.7	-19.4	5.3	**8.0**	1.3	11.0	6	-10.0	**8.0**	9.1	15.1
	TOT dom	34	-23.7	**-21.4**	1.0	-19.4	4.5	**6.8**	1.4	11.0	12	-10.0	**4.3**	7.8	15.1
	deer	3	-22.6	**-21.9**	0.6	-21.4	4.8	**5.5**	0.9	6.5	1	10.4	**10.4**		10.4
NZ (5)	cattle	1	-21.5	**-21.5**		-21.5	6.0	**6.0**		6.0	1	0.1	**0.1**		0.1
	sheep/goat	1	-22.7	**-22.7**		-22.7	7.4	**7.4**		7.4	0				
	TOT herbiv	2	-22.7	**-22.1**	0.9	-21.5	6.0	**6.7**	1.0	7.4	1	0.1	**0.1**		0.1
	pig	1	-19.7	**-19.7**		-19.7	6.8	**6.8**		6.8	0				
	TOT dom	3	-22.7	**-21.3**	1.5	-19.7	6.0	**6.7**	0.7	7.4	1	0.1	**0.1**		0.1
	aurochs	1	-23.3	**-23.3**		-23.3	4.5	**4.5**		4.5	1	9.5	**9.5**		9.5
	deer	1	-23.5	**-23.5**		-23.5	5.0	**5.0**		5.0	1	7.9	**7.9**		7.9

complex.[4] Carbon and nitrogen isotopic data is consistent with that cattle is mainly consumed in addition to some resources enriched in ^{15}N and ^{13}C, particularly for humans with the highest nitrogen isotopic ratios. Pigs are good candidates and probably were also significantly consumed. Sulphur isotopic values do not disagree with a consumption of pigs, though they do not allow concluding. Moreover, a previous study suggested that young domestic animals could be consumed in addition to cattle, providing higher $\delta^{15}N$ values due to the nursing effect (Rey et al. 2017). Indeed, slaughter patterns in regional sites of dairy production suggest that young herbivores could be killed and consumed though they were still breastfeeding or had just been weaned. This would yield a higher trophic level than for adults (e.g. Balasse et al. 2012; Balasse et al. 2000). Young pigs could also be eaten for their tender meat (Hachem et al. 2014). Several juvenile pigs have been found at GLN and have been analysed in this study, together with young herbivores from BMT. However, their values are not distinct from those of adults (Online Resource 3) and consequently no distinction can be made between the consumption of young and older animals. The highest nitrogen values reported here is therefore not associated to an increased consumption of young animals. On another note, considering the isotopic difference between wild and domestic animals, the human isotopic values, in comparison to the wild herbivore values, corroborate the zooarchaeological data, i.e. negligible consumption of these resources.

Another kind of food has been proposed by L. Rey et al. (2017) to explain the high $\delta^{15}N$ values: freshwater resources. In the Paris Basin during the Mesolithic, fishing is well attested through isotopic data and artefacts (e.g. Naito et al. 2013; Vigne et al. 1989). Whereas during the Neolithic, farming seems to have replaced marine fishing in some Atlantic European coastal sites (e.g. Richards et al. 2003), there is varied evidence available elsewhere, even if it is not highlighted by isotopic data (Robson et al. 2019). In the Paris Basin,
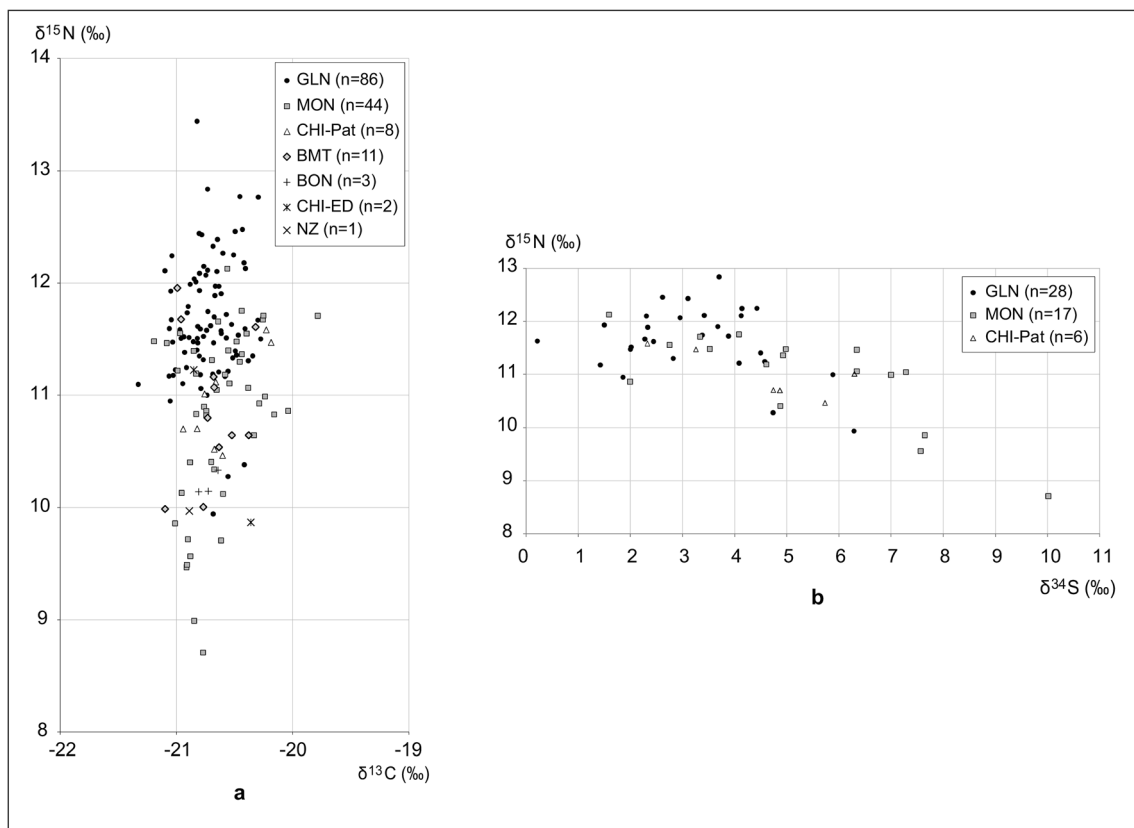
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[4] As the different food sources are not known, the authors chose to not apply any mixing model to quantify proportions based on hypothetic food items. Indeed, these models are applied when the isotopic signatures of the different resources are known. Even if a number of uncertainties can be taken into account (Fernandes et al. 2014), there are too many unknowns here. Various resources not identified and which may have different isotopic signatures could have been consumed additionally in varying proportions (e.g. fish, legumes, cereals and wild plants, molluscs, insects, eggs, algae, birds). This would vary the end-members of the model and thus provide completely different results. Tests with hypothetical end-members values were attempted with the Bayesian mixing model FRUITS (Food Reconstruction Using Isotopic Transferred Signals, Fernandes et al. 2014) and gave very varied and non-informative results.

Fig. 5 Distribution of human isotopic values by site for **a** δ^{13}C vs. δ^{15}N and **b** δ^{34}S vs. δ^{15}N

several Neolithic sites (attributed to VSG[5]) have yielded freshwater fish specimens (Clavel 2009; Clavel and Arbogast 2007). There, fishing (mainly of Cyprinidae) probably provided a non-negligible part of protein intake during hot seasons (ibid.). In the Yonne valley, no aquatic remains have been found (except some drilled marine mussel or scallop shells coming from afar and most likely ornamental), but there are no animal remains in general in the tombs of funerary sites, and the excavation conditions of the dwelling sites (old excavations or rescue archaeology) are not conducive to retrieving any fish bones. However, a hook was discovered in one of the tombs of MON (Mon 99–521; Augereau and Chambon 2011, p. 311). Furthermore, some isotopic studies suggest fishing continued in European inland sites, as for example in the Danube Gorges (Jovanović et al. 2015), in Belgium (Bocherens et al. 2007), in Germany (Dürrwächter et al. 2006) and in France (Goude et al. 2013). The isotopic composition of aquatic ecosystems is highly variable and it is necessary to have a local baseline in order to compare human values to the ecosystem (Dufour et al. 1999; Finlay and Kendall 2007; Nehlich 2015; Nehlich et al. 2013). However, in the absence of any local freshwater remains available, we tried to compare our data with those previously published

from contexts as similar as possible to ours (Fig. 11; Online Resource 9). Published aquatic carbon and nitrogen isotopic data are available for Neolithic German sites (Bösl et al. 2006), and Palaeo-/Mesolithic French and Belgian sites (Bocherens et al. 2014; Bocherens et al. 2011; Drucker and Bocherens 2004; Drucker et al. 2016a; Drucker et al. 2016b). Published sulphur isotopic data are available for Mesolithic French and Belgian sites, and for Neolithic sites in the Danube Gorges (Drucker et al. 2016b; Nehlich et al. 2010). Assuming that freshwater fish from the Yonne valley should plot within the same range of values as in the above named studies, isotopically detectable freshwater fish consumption by humans is possible. As a comparison, human data from Noyen-sur-Seine have also been plotted onto Fig. 11: they are considered freshwater fish-consumers, and their carbon and nitrogen isotopic values are similar to those of the humans from the Yonne valley. Sulphur isotopic values are different but are by far more variable.

Another hypothesis has to be considered: the manuring effect of crop cultivation. Although plant consumption is often hidden by the meat signal in bone collagen (Ambrose and Krigbawm 2003; Bocherens 2009; Hedges and Reynard 2007), it is almost unquestionable that plants (crops and wild herbs) were consumed in addition to meat, although it is not possible here to assess the relative proportions by the isotopic

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[5] Villeneuve-Saint-Germain

Table 5 Summary isotopic results (δ13C, δ15N, and δ34S) of human samples by age and sex for each site; M is male, F is female, I is indeterminate sex, indet is indeterminate age and sex

humans

		age and sex classes	N	δ13C (‰) min	mean	SD	max	δ15N (‰) min	mean	SD	max	N	δ34S (‰) min	mean	SD	max
GLN (86)	M	30	-21.1	-20.7	0.2	-20.3	11.2	11.9	0.4	12.8	15	0.2	2.9	1.2	4.4
	F	18	-21.1	-20.8	0.2	-20.4	9.9	11.5	0.5	12.2	10	1.5	3.7	1.6	6.3
	I	19	-21.0	-20.7	0.2	-20.3	11.2	11.6	0.3	12.4	1	2.4	2.4		2.4
	TOT adult	67	-21.1	-20.7	0.2	-20.3	9.9	11.7	0.5	12.8	26	0.2	3.2	1.4	6.3
	1-9	12	-21.3	-20.8	0.3	-20.3	11.1	11.8	0.8	13.4	0				
	5-14	6	-21.1	-20.8	0.3	-20.4	10.3	11.2	0.7	12.2	2	1.9	3.3	2.0	4.7
	TOT children	18	-21.3	-20.8	0.3	-20.3	10.3	11.6	0.8	13.4	2	1.9	3.3	2.0	4.7
	10-19	1	-20.7	-20.7		-20.7	11.7	11.7		11.7	0				
	TOT human	**86**	**-21.3**	**-20.7**	**0.2**	**-20.3**	**9.9**	**11.7**	**0.6**	**13.4**	**28**	**0.2**	**3.2**	**1.4**	**6.3**
MON (44)	M	11	-21.0	-20.5	0.2	-20.2	9.9	11.1	0.6	11.8	6	3.3	5.5	1.7	7.6
	F	18	-21.2	-20.8	0.2	-20.2	9.6	10.9	0.5	11.6	8	2.0	5.1	2.0	7.6
	I	4	-20.6	-20.4	0.3	-20.0	9.7	11.0	0.9	11.7	1	3.5	3.5		3.5
	TOT adult	33	-21.2	-20.6	0.3	-20.0	9.6	11.0	0.6	11.8	15	2.0	5.1	1.8	7.6
	1-9	3	-20.6	-20.6	0.0	-20.6	10.1	11.1	1.0	12.1	1	1.6	1.6		1.6
	5-9	3	-20.9	-20.5	0.6	-19.8	9.5	10.9	1.2	11.7	0				
	10-14	2	-20.9	-20.9	0.1	-20.8	8.7	9.2	0.7	9.7	1	10.0	10.0		10.0
	TOT children	8	-20.9	-20.6	0.4	-19.8	8.7	10.6	1.2	12.1	2	1.6	5.8	5.9	10.0
	10-19	2	-20.9	-20.9	0.0	-20.9	9.0	9.3	0.4	9.5	0				
	indet	1	-20.7	-20.7		-20.7	10.3	10.3		10.3	0				
	TOT human	**44**	**-21.2**	**-20.6**	**0.3**	**-19.8**	**8.7**	**10.8**	**0.8**	**12.1**	**17**	**1.6**	**5.2**	**2.3**	**10.0**
CHI-Pat (8)	M	3	-20.8	-20.4	0.4	-20.2	11.0	11.4	0.3	11.6	3	2.3	4.0	2.1	6.3
	F	2	-20.8	-20.8	0.1	-20.7	10.7	10.9	0.3	11.1	1	4.7	4.7		4.7
	I	2	-20.9	-20.8	0.2	-20.6	10.5	10.6	0.1	10.7	2	4.9	5.3	0.6	5.7
	TOT adult	7	-20.9	-20.6	0.3	-20.2	10.5	11.0	0.4	11.6	6	2.3	4.5	1.5	6.3
	5-9	1	-20.7	-20.7		-20.7	10.5	10.5		10.5	0				
	TOT human	**8**	**-20.9**	**-20.6**	**0.3**	**-20.2**	**10.5**	**11.0**	**0.4**	**11.6**	**6**	**2.3**	**4.5**	**1.5**	**6.3**
BMT (11)	indet	11	-21.1	-20.7	0.3	-20.3	10.0	10.9	0.7	12.0	0				
	TOT human	**11**	**-21.1**	**-20.7**	**0.3**	**-20.3**	**10.0**	**10.9**	**0.7**	**12.0**	**0**				
CHI-ED (2)	5-14	1	-20.4	-20.4		-20.4	9.9	9.9		9.9	0				
	indet	1	-20.9	-20.9		-20.9	11.2	11.2		11.2	0				
	TOT human	**2**	**-20.9**	**-20.7**	**0.4**	**-20.4**	**9.9**	**10.6**	**0.9**	**11.2**	**0**				
BON (3)	adult I	2	-20.8	-20.7	0.1	-20.6	10.1	10.2	0.1	10.3	0				
	indet	1	-20.7	-20.7		-20.7	10.1	10.1		10.1	0				
	TOT human	**3**	**-20.8**	**-20.7**	**0.1**	**-20.6**	**10.1**	**10.2**	**0.1**	**10.3**	**0**				
NZ (1)	adult M	1	-20.9	-20.9		-20.9	10.0	10.0		10.0	0				
	TOT human	**1**	**-20.9**	**-20.9**	**0.1**	**-20.9**	**10.0**	**10.0**	**0.1**	**10.0**	**0**				
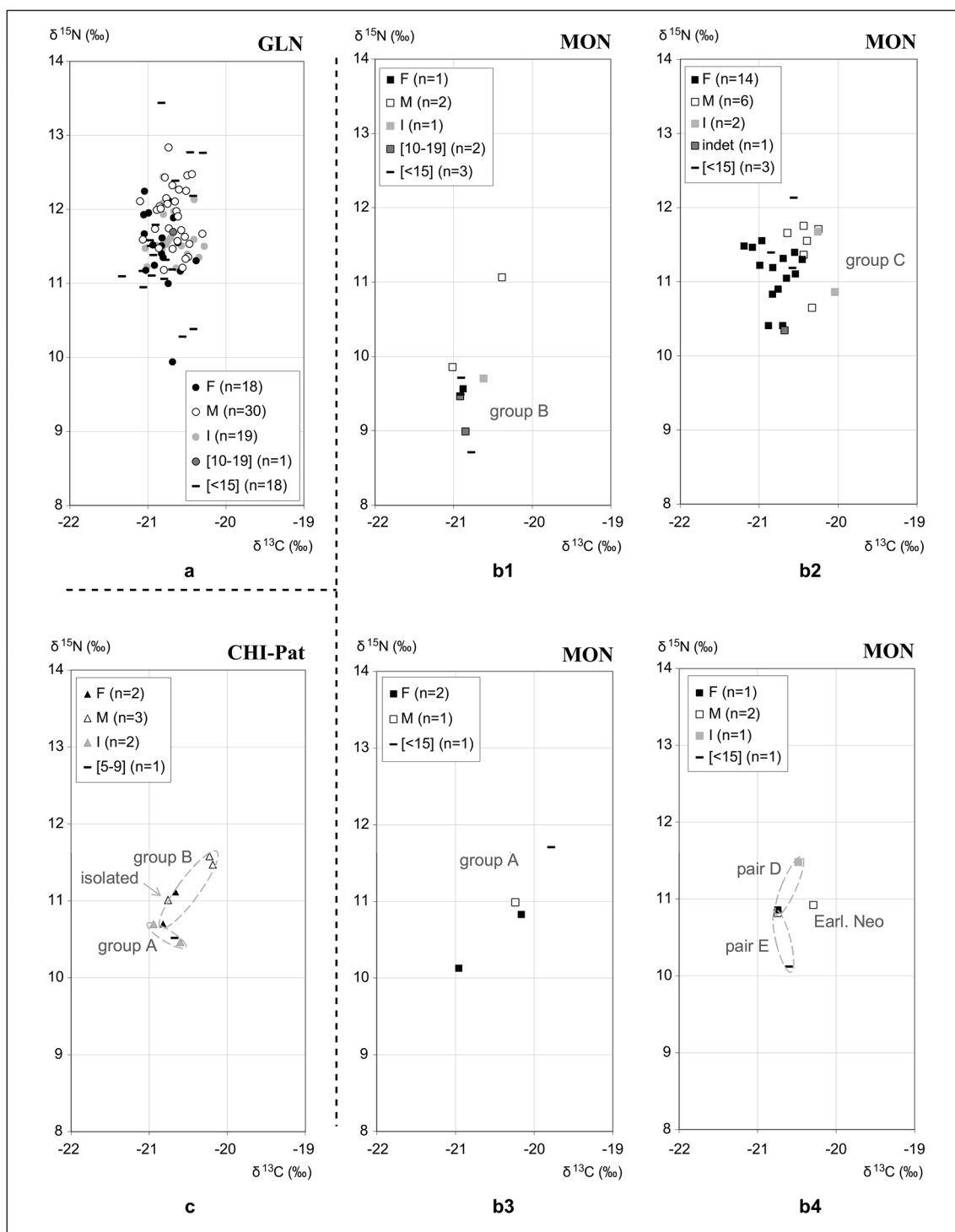
Fig. 6 Distribution of human $\delta^{13}C$ and $\delta^{15}N$ values by age and by sex at **a** GLN, **b** MON, for each group separately (b1 = group B, b2 = group C, b3 = group A and b4 = pair D, pair E and early Neolithic burial) and **c** CHI-Pat

study. The combination of isotopic methods to other techniques as dental calculus analyses or paleoparasitology could bring more information about individual plant consumption (e.g. Goude et al. 2018). Anyway, cereal cultivation with animal manure can elevate the $\delta^{15}N$ values and register higher

nitrogen isotopic ratios in humans eating manured crops than herbivores eating unmanured forage (Bogaard et al. 2013; Gron et al. 2017). Cases of intensive crop manuring were highlighted in several Neolithic sites in Europe and generally used to lead to overestimate the contribution of animal protein
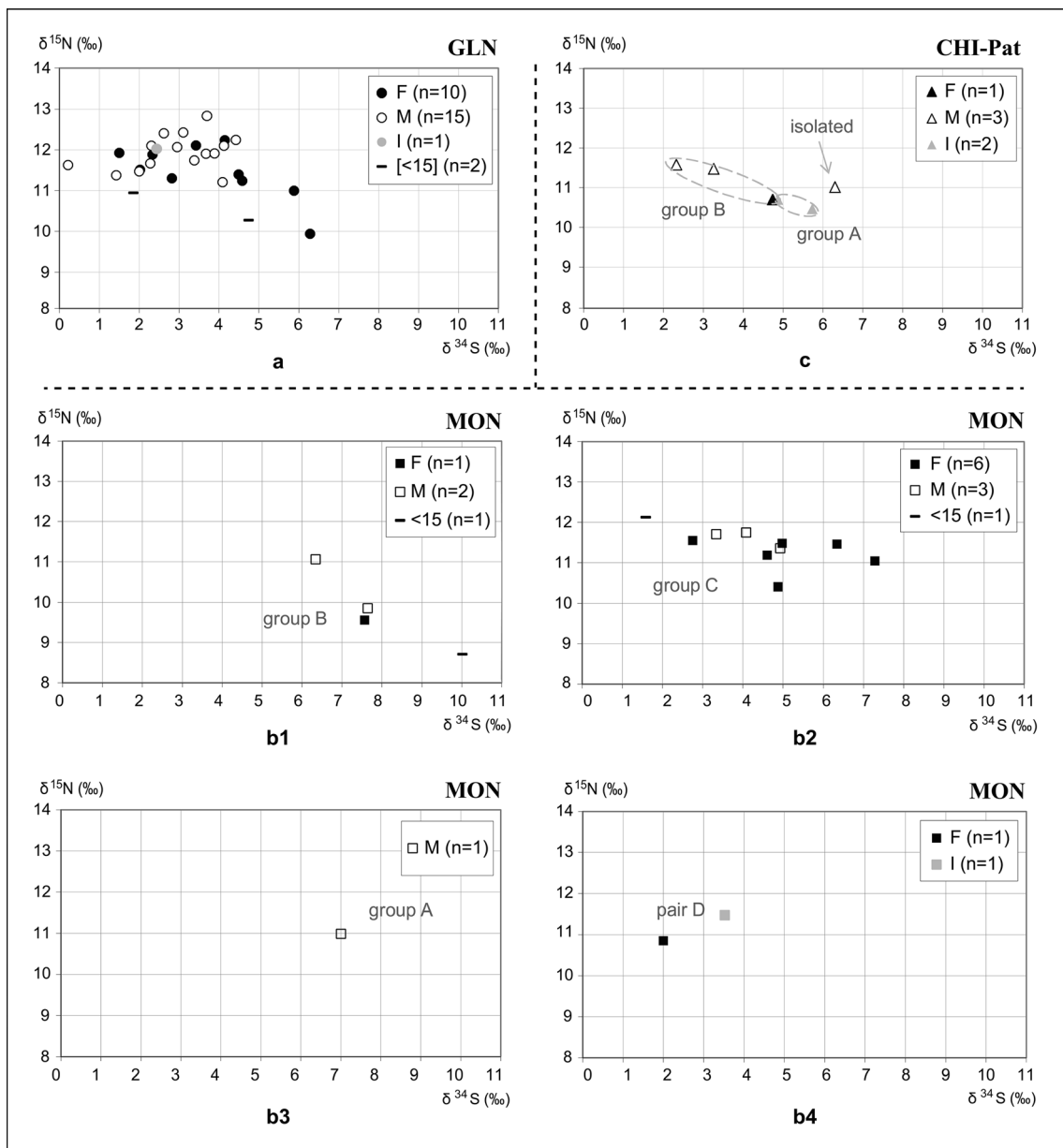
Fig. 7 Distribution of human δ^{34}S and δ^{15}N values by age and by sex at **a** GLN, **b** MON, for each group separately (b1 = group B, b2 = group C, b3 = group A and b4 = pair D, pair E and early Neolithic burial) and **c** CHI-Pat

to the human diet (ibid). No archaeobotanical remains were found in the Yonne valley to address this issue but this hypothesis could explain the widest δ^{15}N offset registered between some humans and herbivores (especially at GLN). This would testify to different plant cultivation strategies between sites.

Finally, the relationship observed here between humans and animals seems to show that humans occupy a particularly high level in the local food chain, especially at GLN, when compared to what is known for elsewhere in Europe in the Neolithic period (Fig. 10; see also Online Resource 10 and 11). As a comparison, an

important consumption of animal protein was also proposed, with a possible consumption of aquatic resources, in the nearby sites of Pontcharaud 2 (Auvergne) and Gougenheim (Alsace; Goude et al. 2013; Goude et al. 2014; see Fig. 10 and Online Resource 10). The neighbouring and contemporaneous Cerny site of Balloy "Les Réaudins" (Seine-et-Marne) presents a similar position of the humans in the trophic chain, maybe reflecting a regional specificity (Herrscher et al. 2012). In this region (Paris Basin or more largely North of France), people either must have had a more protein-rich diet (more carnivorous) than several other Neolithic populations or consumed a greater quantity of
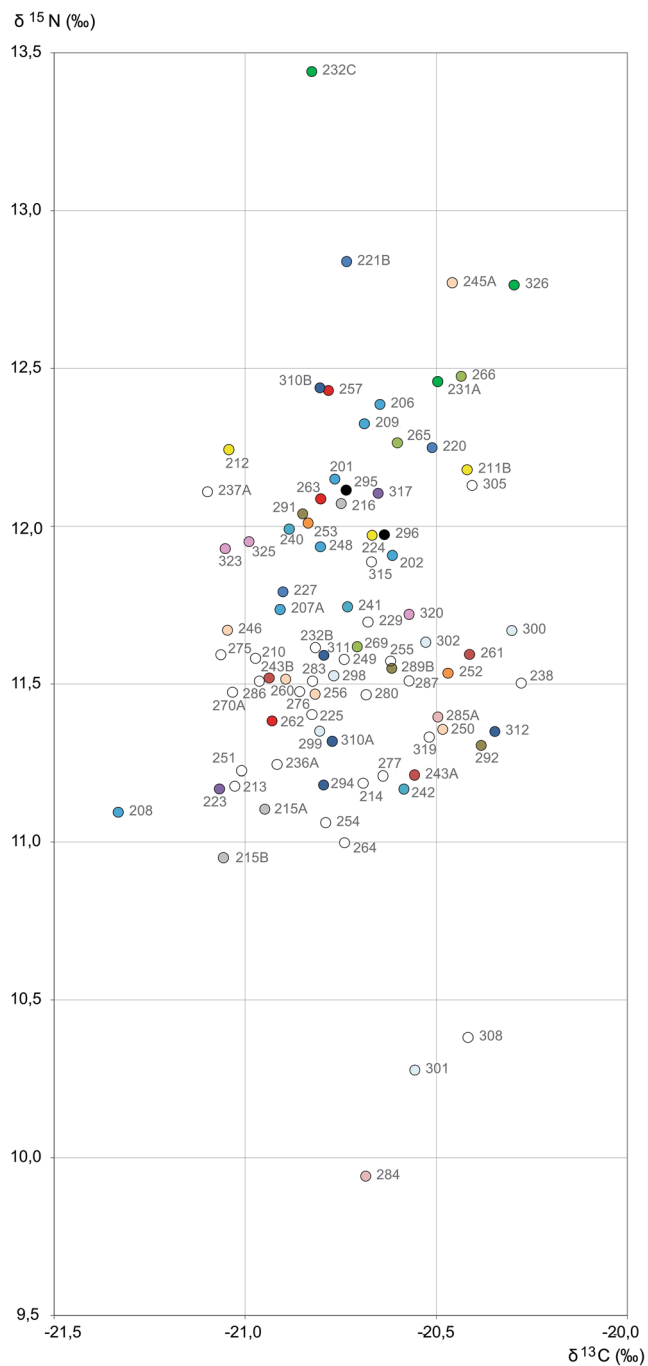
Fig. 8 Repartition of $\delta^{13}C$ and $\delta^{15}N$ values of humans from GLN relatively to their spatial proximity. Each colour brings together individuals buried on the side

proteins enriched in ^{15}N, such as pig or fish, or even a diversified plant cultivation using manuring. Complementary analyses on organic residues of pottery, dental calculus or paleoparasitology could bring new elements to assess the local human diet. Anyway, the present result highlights a regional specificity already identified through the archaeological data.

Diet and social organisation

Although there are no animal isotopic data for every site considered in this study, and despite the slight variations observed, the overall dispersion of values is more or less consistent for all the sites analysed here: domestic herbivores, pigs, and wild herbivores have distinct specific values in the three sites, with nearly the same mean between sites. These animal samples can therefore be used as the local baseline for all the humans (of each site). If animals from GLN display values slightly different to those from BMT (mainly for $\delta^{13}C$ and $\delta^{15}N$, and for pigs' $\delta^{34}S$), the human isotopic ratios are even more distinct between them, meaning the human variability can be due to dietary distinctions, different origin/mobility (with different isotopic background), or chronological effects. Subsequently, the isotopic variability of humans identified between sites can represent in part the animal variability between sites, but could probably also represent dietary or mobility specificities linked to social or cultural reasons. In fact, the various sites studied here seem to have different functions (enclosures, cemeteries, small funerary complexes) and refer to different cultures and periods. However, it is not known how these influences could affect dietary practices and isotopic signatures of these groups of people.

This study does not highlight any correlation between the isotopic data and the cultural attribution of the sites, nor with their nature or function. By seeking a comparison in the surrounding area, the site of Balloy "Les Réaudins", attributed to the Cerny culture, has been analysed for C and N isotopic ratios in bone collagen (Herrscher et al. 2012). Contrarily to the sites of NZ and CHI-Pat, also attributed to the Cerny culture, this one is composed of monumental and highly organised structures, illustrating a hierarchization of funerary spaces (Thomas 2011, p. 189–258). The comparison between Cerny's monumental structures[6] and open-air funerary structures of the same culture may be particularly helpful to track social distinctions. However, the humans isotopic values of Balloy are in the same range as CHI-Pat and NZ (and in the same range as all the other sites of the Yonne valley), which does not reveal any differences in terms of diet (at least for aspects of the diet reflected by the isotopic analyses; Fig. 10). Balloy and CHI-Pat, both constituted by alignments of burials, particularly share very close average values. The farther and more recent sites of La Villette and Bercy (Paris Basin), attributed to a different Chassean type (*Chasséen septentrional*) than those of the Yonne valley, seem less relevant for a cultural comparison here. Regardless, they show on average lower values than humans from the Yonne valley (Bocherens et al. 2005). The link with the chronology is more complex due to problems with radiocarbon dates performed on human bone

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[6] Thus far, Balloy is the only site among Cerny's monumental structures that has had isotopic analyses performed.

(Augereau and Chambon 2011, p. 339–353; Meunier 2007) and to the difficulties of attributing a specific cultural phase when the archaeological material is poorly represented. Furthermore, sequencing the sites and cultural groups in the Yonne valley is still one of the big unresolved issues of archaeology in the region (Augereau et al. 2014; Chambon et al. 2013). So, individual dating, together with cultural attribution of the sites and individual groups, have to be considered carefully. Notwithstanding, the isotopic differences identified between sites and areas clearly distinguish several human groups, living separately in space or in time (depending on the sites), even if they were exchanging, or transmitting, knowledge and objects. The proximity between GLN and MON group C, given by both archaeological and isotopic data, is particularly noteworthy. This reflects the already highlighted complexity (and specificity) of this region, considering the multiple cultural influences encountered and the difficulty to identify well-defined groups (from an archaeological point of view, not isotopic or dietary, that is not known).

Concerning the relationship between burial practices and isotopic data, only some trends stand out at GLN: adult subjects buried in alcoves have similar δ^{13}C and δ^{15}N values (sample size is not appropriate for statistical analysis; Fig. 8, light blue dots). Note that these individuals are all adult males (the two children in alcove have lower values) and are grouped in the south-western part of the cemetery. Alcove burials refer to the RRBP culture and may constitute a coherent socio-cultural group (Rottier 2007). In this site, we have also highlighted isotopic similarities between individuals buried in tombs grouped together (Fig. 8), without these individuals sharing other funerary or biological characteristics (genetic studies in progress seeking for potential parental connections). These groupings could reflect social relationships within the population and would rather suggest a horizontal organisation of the society. On another side, the man GLN 221B presents the highest δ^{15}N value of the overall GLN adult group and occupies the biggest grave with a "central" place in the cemetery (there seems to be a peripheral organisation around his tomb; no specific grave goods were associated; Online Resource 2a). This man could have had a special status among the group, with a specific diet reflected through isotopic data. However, there is no other evidence to suggest a social stratification (neither at GLN nor in other sites) and this argument alone is not sufficient to support such a hypothesis. In overall, there is no general correlation between the burial parameters and the isotopic data inside and between groups, despite the great variability of the graves. The latter could have reflected socio-cultural disparities that could be expressed through food, as certain types of tombs are linked to particular cultural trends (e.g. alcoves referring to the RRBP, Balloy tombs referring to the Cerny). The lack of any significant link with burial practices (no specific isotopic signatures for the different types of graves) raises once again questions about the relationship between funerary practices and archaeological culture, on the one hand, and between archaeological culture and population on the other hand, particularly in this context. Moreover, the food does not always reflect social structure in the same way, particularly through isotopic data. But when some disparities are visible, we can wonder if food is rather determined, and how, by the region, the culture, the social status (at the intra-group level), the chronology or another parameter.

Then, one of the most striking results comes from the comparison of the isotopic datasets of males and females. Specific sex-based behaviours have been highlighted and echo a previous local study for the Neolithic (Rey et al. 2017). Isotopic distinctions between males and females from the same site, together with the similar isotopic values of some individuals buried besides one another or sharing a similar funerary process (for the particular group of GLN), suggest a visible social division, in particular between sexes. Taking the three proxies together, a principal component analysis (PCA) clearly highlights the isotopic difference recorded between human males versus females by site (Fig. 12). It is worth noticing that the isotopic differences registered between men and women are not the same as those recorded between sites. On the one hand, men and women have different C, N and S average isotopic ratios; on the other hand, only the N and S average isotopic ratios are varying between sites while the C average isotopic ratios are homogeneous. These differences do not have the same explanation and could be linked to differential consumption of resources or environmental specificities (that is to say food provenience or human mobility). In average, men could have consumed more resources enriched in ^{15}N and ^{13}C and depleted in ^{34}S than women, who could have consumed more resources depleted in ^{15}N and ^{13}C and enriched in ^{34}S. On the other hand, some sites (namely GLN, MON group C and CHI-Pat, in a lesser extent) display higher N, lower S and similar C isotope ratios than other sites (namely NZ, BON and CHI-ED). Although there is no isotopic data available locally for freshwater fish remains, the comparison with published S values from Belgium (δ^{34}S between − 5 and + 4‰; Fig. 11b) shows that if local values were similar, a greater consumption of fish could explain, for example, higher δ^{15}N and lower δ^{34}S values registered in some sites compared to others. The C isotopic signature of fish varies between the sites of comparison (Fig. 11a) and could be similar, on average, to that of humans from the Yonne valley, inducing negligible variation between sites. Furthermore, as S isotopic values are highly variable from one environment to another and considering the great variability among the humans, it is quite possible that individuals with different values have lived in different environments or travelled between various environments in life. An ongoing study on strontium isotopes will bring new elements to discuss the individual mobility of these populations of the Yonne valley.
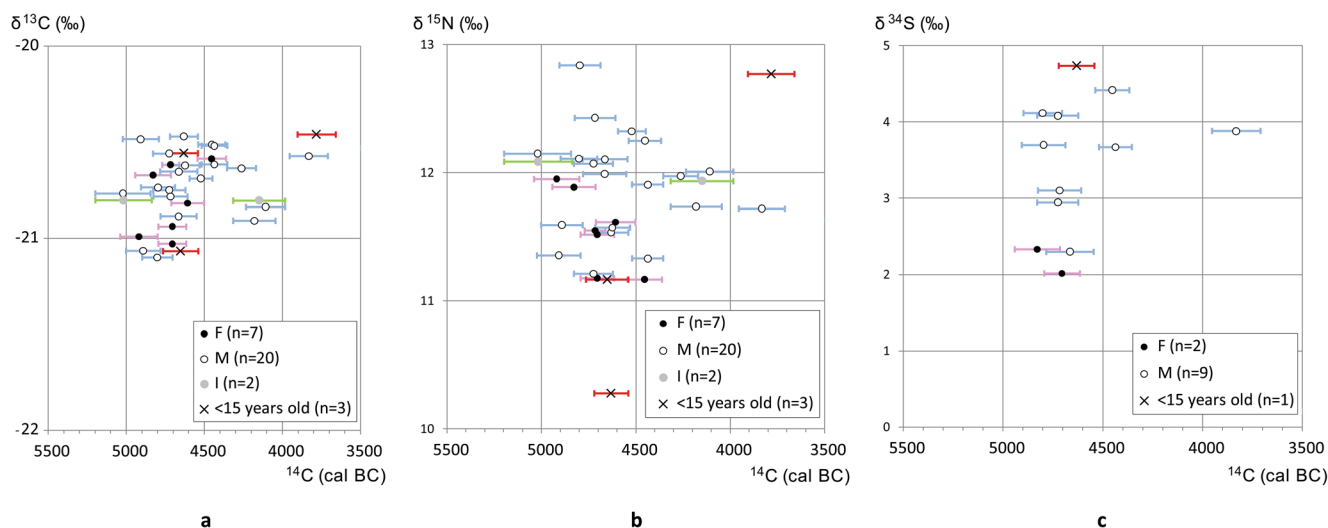
Fig. 9 Distribution of isotopic values relatively to chronology (from 5200 to 3800 cal BC) of the human group of GLN. **a** δ¹³C, **b** δ¹⁵N and **c** δ³⁴S

Finally, several hypotheses can be proposed to explain the individuals isotopic variations: (1) a differential protein intake from different animal species, (2) a differential protein intake from the same species but raised (or sometimes hunted) in different areas or with different fodder (given the BMT vs. GLN distinction), (3) a differential consumption of plants (namely legumes, as protein-rich as meat), which have lower $\delta^{15}N$ and $\delta^{13}C$ than animals but whose $\delta^{34}S$ values are

unknown, and/or (4) different origins, even tenuous, or different patterns of mobility. With regard to the differences observed between individuals, which are all in all subtle, these could also be explained by differences in the part of the food-stuff being consumed (different pieces of meat, like offal vs. muscle; different parts of plants, like leaf vs. seed, different nutritional composition of plant as legume vs. cereal), or even by some physiological differences (Petzke and Fuller 2012),

Fig. 10 Regional comparison of isotopic values available in literature for the Neolithic period. All the data are detailed in Online Resource 8 and come from the following publications: (1) this study; (2) Balasse 1999; Herrscher et al. 2012; (3) Bocherens et al. 2005; (4) Goude 2014; (5) Goude et al. 2013; (6) Goude et al. 2014; (7) Oelze et al. 2011; Nehlich et al. 2009; Dürrwächter et al. 2006
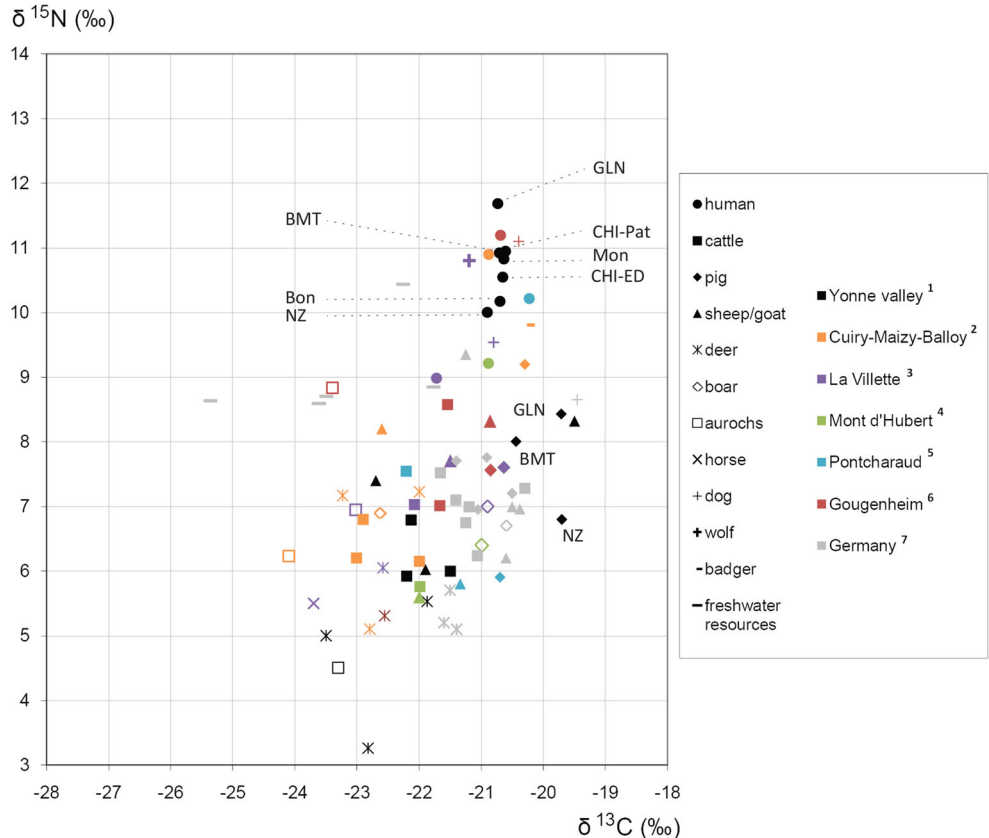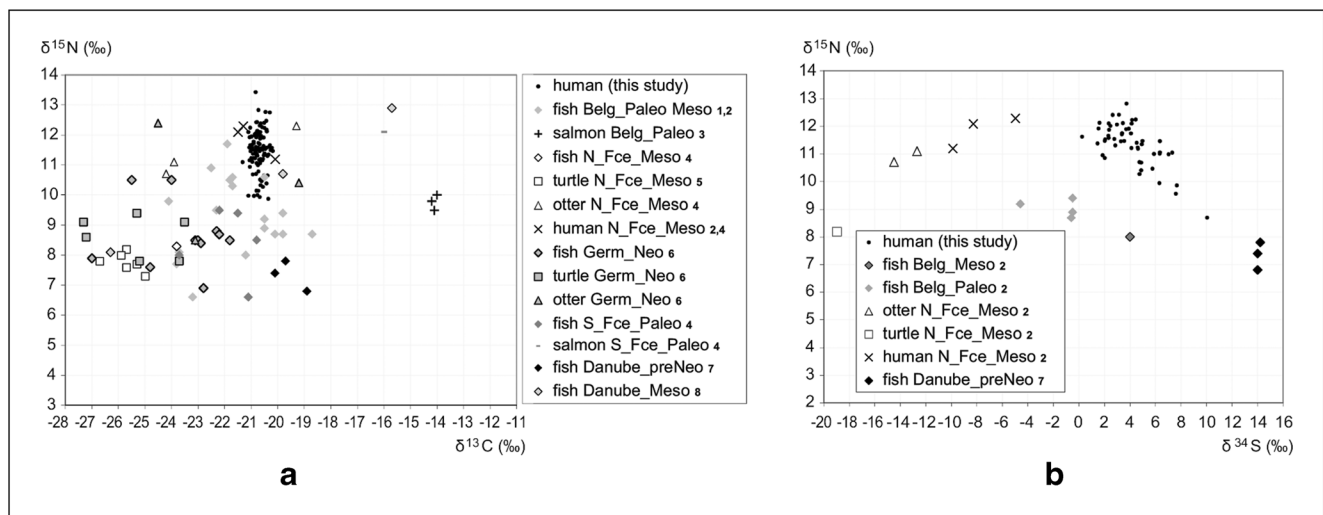
Fig. 11 Comparison of human isotopic values with available data for freshwater resources for **a** $\delta^{13}C$ vs. $\delta^{15}N$ and **b** $\delta^{34}S$ vs. $\delta^{15}N$. Abbreviations are used for the sites where the data comes from. *Belg*, Belgium; *N_Fce*, Northern France; *S_Fce*, Southern France; *Germ*, Germany; *Paleo*, Palaeolithic; *Meso*, Mesolithic; *preNeo*, pre-Neolithic; *Neo*, Neolithic. All the data are detailed in Online Resource 9 and come from the following publications: (1) Drucker et al. 2016a; (2) Drucker et al. 2016b; (3) Bocherens et al. 2014; (4) Drucker and Bocherens 2004; (5) Bocherens et al. 2011; (6) Bösl et al. 2006; (7) Nehlich et al. 2010; (8) Bonsall et al. 1997

rather than by different food items consumed. Additional studies (especially on teeth and residues in pottery) would provide useful information on the different food sources such as plants, dairy products and fish to better characterise the local baseline for the human diet. Primarily, it will be necessary to measure the isotopic signature of freshwater fish specimens in order to refine, in particular, the question of the sex-related and between sites dietary variations.

Whatever the cause of these differences, they represent in themselves social or cultural distinctions, maybe also linked to different mobility patterns, between individuals or groups of individuals. Indeed, the isotopic difference between the sexes could illustrate a sexual division of labour or a social separation of status. In the Neolithic of the broader region, sexual division was already visible in biological studies (Villotte and Knüsel 2014) or associated with grave goods or funerary structures, like in the Cerny culture (Thomas 2011) or at Le Vigneau (Touraine, Coutelas et al. 2015). However, it is relatively new to distinguish such a division through diet, revealed by isotopic analyses, though it has been suggested for other

Fig. 12 Principal component analysis of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ together distinguishing human genders, all sites considered
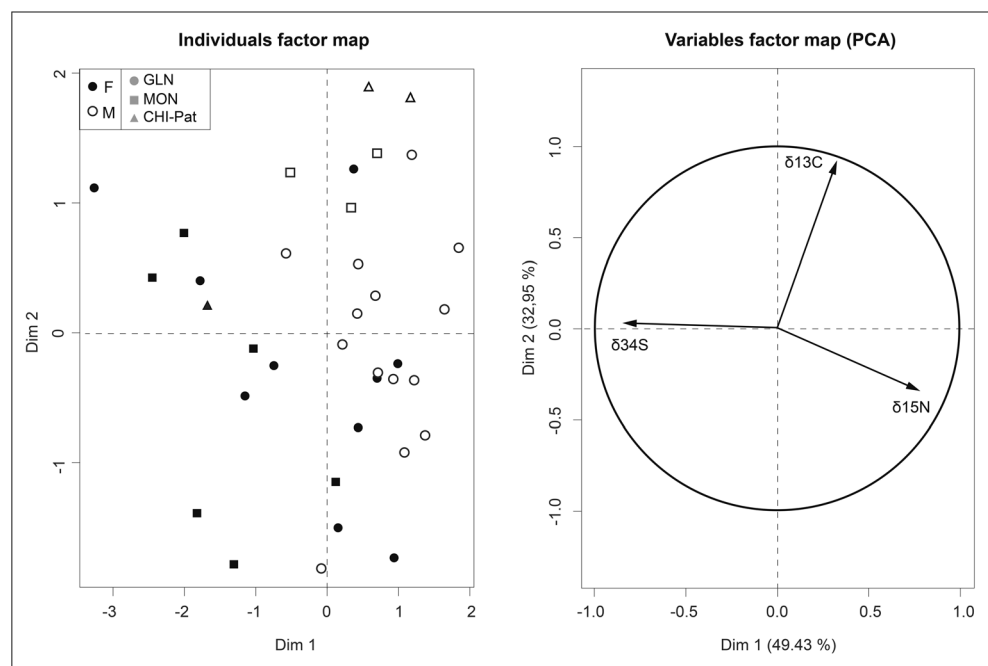
contexts (Ambrose et al. 2003). Compared to other sites where males and females clearly exhibit distinct status through goods (e.g. Thomas 2011, 2014), here, burial criteria do not systematically distinguish sexes. Moreover, isotopic values are not clearly distinct between males and females but rather display a slight tendency. This probably reflects a population broadly sharing the same food supplies and usage of, but in different proportions and/or cooking modes, either linked to their social status, or to their activities. The latter may also involve different mobility pattern with some people travelling or living between different areas, which would lead to averaging different final values than people not moving so much. In this regard, no difference in variability was observed between men and women, which does not allow to propose a mobility scheme towards either a patrilocal or matrilocal system. However, a difference in variability between the sexes seems to be visible during the period between 4800 and 4500 cal BC (Fig. 9), which is known to become more complex and socio-culturally diverse in this region (Chambon et al. 2013; Constantin et al. 1997; Thomas 2011). Nevertheless, the difference in variability is due to only a few samples and may be due to sampling bias.

Finally, regarding the children's isotopic pattern, the extreme values often reported in this study may express the particular nutritional needs required by their growth (e.g. de Luca et al. 2012). Physiological parameters (relative to growth and growth conditions such as health status) and their possible influence on bone isotopic ratios are somewhat unknown (e.g. Herrscher et al. 2017; Mekota et al. 2006). This pattern may also represent a particular diet for children, by social choice, or still a weaning signal, providing them different isotopic values to adults.

Conclusions

This study confirms previous results obtained at GLN and brings new elements enabling a more in-depth discussion on regional dietary behaviours relating to cultural/ functional inter-site variability and regional specificities (at an individual and population scale). Firstly, the data show that the human diet (protein part) is mainly based on cattle and pig, with a possible additional consumption of freshwater fish. The proportion of meat and fish could vary between sites. The multi-proxy analysis was very useful and pointed out new information relative to the human subsistence economy: for example, the diversified exploitation of pigs between sites. However, a direct analysis of additional food sources, and particularly of fish, is necessary to answer questions about their consumption. Secondly, some differences were highlighted between the humans of the different sites. Humans from GLN presented the highest trophic positions while those from NZ

and BON were among the lowest. Nevertheless, there is no correlation between the isotopic values and the function of the sites, nor with their cultural attribution. Certain isotopic patterns seem to correlate with chronology: on the one hand, the lowest nitrogen isotopic values are found among the MON's group B, one of the most recent group, on the other hand, greater nitrogen isotopic variability seems to have been measured for the beginning of the Middle Neolithic (around 4500–4800 cal. BC). Thirdly, sex-related isotopic distinctions are clearly identified, although slight, and reflect a diet or mobility pattern influenced by sexual division. Such a division is known, for the Neolithic period, through funerary behaviours (although not in the Yonne valley, where there is no real sexual difference in funerary treatment, but rather preferences in ornaments, tools, or type of grave, without exclusivity for one sex or another). However, it is relatively new to detect it in food. Furthermore, given the still small isotopic contrast between males and females, they could have consumed the same type of resources, but in different formats (proportions or cooking modes for example), related to their respective social status or their activity. In other words, there was probably no prohibited food for either sex, but they could not have shared the same meals, the same preparations, or they maybe have had different patterns of mobility. This point may particularly highlight the emerging complexity coming out from the new Neolithic lifestyles. Finally, the important variety of funerary behaviours encountered in the Yonne valley is not reflected by the isotopic patterns, which are overall homogeneous. However, some particular burial ensembles share similar isotopic values revealing inside group proximities (e.g. GLN burial groupings, CHI-Pat groups).

This is the most significant dataset to date for Neolithic France in terms of sample size from a limited area. It has allowed the identification of inter- and intra-site differences, at the wide scale of a well-defined region. The isotopic comparisons with neighbouring sites emphasise once again the regional peculiarity of the Yonne valley: a high trophic level for humans (with a possible consumption of fish) and sex differences not highlighted elsewhere. From these results, a food model can be proposed based on a rather horizontal stratification of society, with differences between the sexes and between groups of individuals sharing similar or close burials (maybe related to social links), and probably different livestock management styles between neighbouring groups. Comparison of these results with, in particular, new analyses from the monumental Cerny structures (whose social organisation appears hierarchical) will enable further research on this question.

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