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Bronze Age innovations and impact on human diet: A multi-isotopic and multi-proxy study of western Switzerland

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Abstract

The archaeological Bronze Age record in Europe reveals unprecedented changes in subsistence strategies due to innovative farming techniques and new crop cultivation. Increasing cultural exchanges affected the economic system. The inhabitants of Switzerland played a pivotal role in this European context through relationships with the Mediterranean, the High and Middle Danube regions and the Alps thanks to the area's central position. This research aims to reconstruct, for the first time in Switzerland, human socio-economic systems through the study of human diet, herding and farming practices and their changes throughout the Bronze Age (2200-800 BCE) by means of biochemical markers. The study includes 41 human, 22 terrestrial and aquatic animal specimens and 30 charred seeds and chaff samples from sites in western Switzerland. Stable isotope analyses were performed on cereal and legume seeds (δ^{13} C, δ^{15} N), animal bone collagen (δ^{13} C_{coll}, δ^{15} N, δ^{34} S), human bone and tooth dentine collagen ($\delta^{13}C_{coll}, \delta^{15}N$,) and human tooth enamel ($\delta^{13}C_{enamel}$). The isotopic data suggest a) an intensification of soil fertilization and no hydric stress throughout the Bronze Age, b) a human diet mainly composed of terrestrial resources despite the proximity of Lake Geneva and the Rhone river, c) a diet based on C_3 plants during the Early and Middle Bronze Age as opposed to the significant consumption of ¹³C-enriched resources (probably millet) by individuals from the Final Bronze Age, d) no important changes in dietary patterns throughout an individual's lifespan but a more varied diet in childhood compared to adulthood, e) no differences in diet according to biological criteria (age, sex) or funerary behavior (burial architecture, grave goods).

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Introduction

The Bronze Age (2200–800 BCE) is a period of important social and economic growth. Large cultural and ethnic traditions, with distinctive features, arose in Europe for the first time [1], developing in a larger, connected framework. This study explores the biochemical aspects of this phase of economic and social changes, which influenced every aspect of human life, including lifestyle, subsistence strategies and food habits. The reconstruction of farming practices, land use and, consequently, dietary behaviors, which directly affect prehistoric communities' way of life, contribute to the understanding of these complex socio-economic dynamics.

Archaeological evidence suggests that Bronze Age communities lived on agriculture, animal husbandry and wild plant harvesting [2, 3]. From the Bronze Age onwards, the south of central Europe was characterized by the expansion of wide open spaces, reducing the areas covered by permanent forests [4]. These open areas were cultivated or used as pasture for livestock. The spread of the cultivated land led to the exploitation of less fertile soil, especially during the Final Bronze Age, a time of demographic growth [5]. To increase agricultural production, significant innovations were introduced and a number of new crops were cultivated, adding to the diversity of food resources [2], especially in areas characterized by poor soils or during periods of drought [6]. New management systems like crop rotation and the use of manure as well as new farming strategies which involved controlling the hydric systems developed, leading to a more intense and rationalized agricultural activity [7, 8].

The Bronze Age was also characterized by the increased mobility of goods and people, in long-distance exchange networks. Indeed, one of the main driving forces at the origin of large cultural complexes was the exchange of metal and other raw materials across Europe from only a few key areas and, consequently, long-distance trade transport [9, 10]. The distribution of bronze metallurgy, which requires both copper and tin, uncommon and unevenly distributed ore deposits, led to heightened relationships at both the local and regional levels and to the multiplication of exchanges at the European level [11–13]. Indeed, a new globalized world of interconnectivity took shape and the movement of people was at the base of this phenomenon, as has been confirmed by aDNA research [14–16].

In this context of greater European networking and socio-economic development, the archaeological record shows that Switzerland played a major role because of its strategic position for exchanges with both central and southern Europe [12, 17, 18]. Swiss archaeological sites from this period showcase a mixture of local traditions and influences from surrounding societies [18, 19]. Consequently, Switzerland is an exceptional observatory for the reconstruction of dietary patterns, and subsistence strategies during the Bronze Age. The region is characterized by high environmental variability: the rapidly changing biotopes have different natural resources, making this territory even more interesting for the study of subsistence strategies.

The aim of this research was to investigate how dietary habits and farming practices changed during the Bronze Age through a multi-isotopic approach on human, animal and botanical remains. Isotopic analyses accomplished on the entire trophic chain can clarify a) how agricultural practices evolved over time, b) whether manuring occurred or not, c) crops hydric condition and d) changes in dietary patterns. Some large European necropoles of the Bronze Age, such as Arano di Cellore, Olmo di Nogara (Italy) [20–22] and Singen-am-Hohentwiel (Germany) [23], have already been the subject of dietary investigations providing key economic details about agricultural practices. The high dietary variability detected in biochemical studies of the Bronze Age of southern Europe [20, 21, 24, 25] has led to further research on the spread of new plant foodstuffs, like C₄ plants, in Europe from the Middle Bronze Age. Switzerland, particularly its western area, offers excellent archaeologically and

chronologically well-documented datasets to explore these types of questions through a multiisotopic and multi-proxy approach.

The Bronze Age of western Switzerland

Placed in the north-western portion of the Alpine mountain chain, Switzerland has a strategic geographic position. It is crossed by a large plateau oriented northeast-southwest, with a rich hydrographic network and several lakes, connecting western and central Europe. In addition, several Alpine passages provide access to southern Europe through northern Italy (Fig 1). At the crossroads of various exchange networks and cultural influences, the Bronze Age of Switzerland (Early Bronze Age: 2200–1500 cal. BCE, Middle Bronze Age: 1500–1300 cal. BCE, Recent and Final Bronze Age: 1300–800 cal. BCE [19]) is characterized by a mosaic of cultural groups, both in time and space (e.g., culture of the Valais [18, 26]). However, current knowledge of this cultural variability is limited by the uneven quality of the archaeological record from the different chronological phases [27].

Despite these limitations, several lines of archaeological evidence point towards an increase in socio-political, economic and technological complexity throughout the Swiss Bronze Age. Funerary practices shifted from cemeteries of individual burials in the Early Bronze Age [28– 30] to grouped graves under tumuli and rare burials with multiple individuals in the Middle Bronze Age [31–33] to mixed necropoles with individual cremations and inhumations at the end of the Bronze Age [34, 35]. Grave goods are present in some burials in all Bronze Age periods: they are limited to bronze artefacts in the Early Bronze Age, while from the Middle Bronze Age pottery occurs sometimes, becoming more frequent during the late Bronze Age. Metal artefacts continue to be present, especially prestige objects like in Middle Bronze Age burials [19, 36] and in the later Bronze Age ones [35]. The increasingly specialized practice of





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metallurgy required exchanges with the south of the Alps, the Danube Basin and the eastern Mediterranean [37–39].

This trajectory of increased complexity and social stratification is difficult to study archaeologically for the later Bronze Age periods because the practice of cremation was introduced in the Middle Bronze Age and became almost exclusive during the Recent and Final Bronze Age in Switzerland, as in most of Europe (e.g., [40]). Exceptions to this pattern are the Final Bronze Age cemeteries from the Lake Geneva Basin and the upper Rhone valley [35]. Indeed, in the Final Bronze Age, both cremations and inhumations were present in south-western Switzerland but cremations seemed more complex in terms of funerary architecture and grave goods, suggesting that they may have been reserved for the elite [35]. This contrasts with earlier periods in the same areas where the small size of the necropoles suggests the existence of small communities with limited social differences.

In parallel with these social and demographic changes, during the Bronze Age, subsistence patterns intensified, involving several aspects of the production economy. Throughout the Bronze Age, the economy was based on agriculture, cattle breeding and woodland exploitation, in substantial continuity with the agro-pastoral economies practiced since the Neolithic [41, 42]. However, in addition to wheat and barley, new varieties of pulses and cereals, such as lentils, beans, spelt and millet were introduced [42–45]. Not only the number of plants increased, but also the production techniques improved and land use changed [42, 46]. There is evidence for the use of crop rotation, fertilization and water supply systems to increase agricultural production [47]. Animal husbandry still played a crucial role in the economy, with an increased dominance of sheep and goats at the end of the Bronze Age, although the main meat supply was provided by cattle [48–50]. Sediment records and strontium isotope analysis performed on animal remains support seasonal movement of livestock to higher elevations [51]. Evidence for a well-organized use of the territory is demonstrated by the exploitation of wetlands around the lakes: wet meadows beyond the reedbeds, in all likelihood seasonally flooded, provided bedding for livestock.

All these increasingly complex agricultural and pastoral activities are apparent in the archaeological record [27, 41]. However, there remain important gaps in current knowledge, such as the timing and geographic patterning of the introduction of these new agricultural and pastoral techniques. In addition, research has focused on archaeobotanical and archaeozoological remains without any direct evaluation of the resources consumed through biochemical analyses. As a result, it is not known whether the new plants were destined for animal or human consumption, whether manuring was routinely applied or not and whether it was used on all crops or only on a select few. Similarly, almost no information is available about hydric conditions and if some crops were preferentially watered compared to others. Multi-proxy isotopic studies on well-dated material have the potential to significantly improve our understanding of Bronze Age economic intensification, changes to human dietary patterns and mobility events.

The cemeteries

Collombey-Muraz, La Barmaz is located in the Valaisan Chablais, around 15 kms from the oriental limit of Lake Geneva (Fig 1). The site has Neolithic and Bronze Age occupations and is composed of the cemeteries of La Barmaz I and II and the settlement of the Refuge hill [18, 30]. The Early Bronze Age necropolis of La Barmaz I (2200–1550 cal. BCE) includes 15 individual burials. Anthropological analyses identified 13 adults of both sexes and two young juveniles (< 5 years old) [30] (S1 Fig). The present study includes only the 13 adults. Seven graves contained exclusively bronze objects as grave goods: awls, spiraled rings, pearls, a double-spiral pendant, a dagger and an axe [18]. The funerary architecture, the ritual and the grave goods do not suggest any social differences between the individuals [30].

The necropolis of Vufflens-la-Ville, En Sency is located in the Venoge valley, about 9 kms northwest of Lausanne, on a small hill used as a gravel pit (Fig 1) [31, 52]. The necropolis was in use from the Middle Bronze Age to the Early Iron Age. A burial stone mound (tumulus) was erected at the beginning of the Middle Bronze Age, covering the central double burial of an elderly woman and a young man. Two other individual burials, one of an older male and the other of a juvenile, overlap with the edge of the burial mound and date to the Middle Bronze Age (S2 Fig). Contemporary with the latter two individual burials, there is a collective grave of four women and two adolescents in a pit on the south-western part of the tumulus (S2 Fig). The collective burial contained more than 80 amber beads, different pendants and a jug. Two male burials without grave goods located on the western part of the site are contemporary with the previous burials and two other cremated burials belong to later periods. In total, 12 Middle Bronze Age inhumations were found [31]. The individual, double, and collective graves contain individuals of different ages and sex. Anthropological studies identified seven adults and five juveniles. The grave goods suggest that this small cemetery was likely reserved for a local elite [31].

The site of Tolochenaz, Le Boiron-La Caroline is situated to the north of Lake Geneva, in the commune of Tolochenaz (Fig 1). The cemetery is split between two lake terraces, ten and 30 meters high each, Le Boiron and La Caroline, both dated to the Final Bronze Age (1050–800 cal. BCE). Tolochenaz was a bi-ritual cemetery, where both inhumation and cremation were performed. Le Boiron was discovered at the beginning of the 19th century and even if more than 70 graves were initially discovered, more recently only 17 burials and 15 cremations have been recorded [34]. In 2009, the exploitation of a gravel pit on the 30-meter high terrace led to the discovery of 20 graves in the La Caroline area [35]: 19 inhumations, mainly in monolithic coffins, and one cremation. One individual (LaC4) differs from the rest for his unique funerary structure. He was buried prone in a straight ditch surrounded by a circular ditch, while all the other inhumations were supine with extended lower limbs in rectangular pits. Several graves delivered funeral artefacts such as an awl, pins, ankle rings, bracelets, rings, amber and glass beads, razors and ceramic vessels (S3 Fig). For this study, ten individuals from La Caroline and six from Le Boiron were included, for a total of 12 adults of both sexes and four juveniles.

Stable isotope analyses to reconstruct human dietary habits

Carbon isotopic ratios allow us to distinguish the environment where humans acquired their resources (e.g., terrestrial vs. aquatic) and the type of plants consumed. The C₃ type plants are typical of a temperate environment (barley, wheat) and they prevail in Switzerland and in all of Europe, whilst C₄ type plants usually reflect an open and warm environment and have higher values (millet, sorghum) [53–55]. The C₄ plants most cultivated by prehistoric communities were millet varieties, mainly *Panicum miliaceum* and *Setaria italica* [20, 21]. Thus, the possibility of detecting C₄ plant intake is particularly relevant to the present study as it may help identify the first C₄ consumers in central Europe. The isotopic ratios of nitrogen are used to determine the trophic level occupied within the food chain. Plants, being at the base of the food chain, present lower δ^{15} N values and as a consequence the δ^{15} N values of predators are higher than that of their prey [56, 57]. Finally, isotopic ratios of sulfur help identify the origin of dietary resources (terrestrial, aquatic and marine) and detect the presence of non-local individuals [24, 58–60].

From one consecutive link of the chain to the next, a fractionation in favor of the heavy isotope occurs. The concentration in heavy isotope increases (¹³C, ¹⁵N, ³⁴S) and the isotope ratios

rise at a rate estimated from 0 to 1% for carbon, from 3 to 5% for nitrogen, and lower than 1% for sulfur [56, 57, 61–63].

By analyzing remains from different sources, the isotopic values for each source are defined. Archaeobotanical studies increasingly refer to experimental studies of traditional agricultural systems [64]. Even though environmental factors [54, 65–67] as well as geographical conditions [68] influence plants' isotopic values, experimental investigations contribute to quantify the impact of anthropic activities on isotopic values, such as the use of manure on agricultural production [69, 70] and the hydric growing conditions [71–73].

Through the isotopic analyses of animal remains, the local isotopic variability of a diet can be determined. When considering wild and domestic species, environmental factors and husbandry practices can be taken into account as these can strongly influence the isotopic values of domestic animals [74]. Moreover, based on herbivore data, it is possible to evaluate the isotopic value of the fodder they consumed. As such, the estimated isotopic ratios from fodder provide a sample of the local vegetation, to which the values for cultivated plants can be compared [75]. Consequently, agricultural practices, such as the use of manure or irrigation systems for cultivated plants, can be evaluated.

The analyses of the human remains were conducted at both the individual and population level. By taking into account bone and teeth, the changes in diet over an individual's lifespan can be traced because bone and teeth have different growth patterns and turnovers [76, 77]. They are both constituted of a mineral, bioapatite, and an organic component, collagen. On the one hand, bone collagen records the protein components of the diet, and its chemical composition reflects the food consumed over the last ca. 15 years of an adult's life [78]. Teeth, on the other hand, record the biogeochemical information of childhood, which can vary between teeth because of their staged development. Finally, dentine collagen provides information on the protein intake at the beginning of an individual's life. In addition to the study of protein intake, it is also possible to study the global energy provided through food (carbohydrates, lipids and proteins not used for protein tissue synthesis) by analyzing tooth enamel carbonates [79–81]. The δ^{13} C value from apatite is dependent on the energy substrate because it is obtained from blood plasma CO2 which mainly comes from metabolizing carbohydrates. Consequently, the δ^{13} C from apatite provides information on food sources consumed that are low on protein content (e.g. cereals), which are difficult to identify when different dietary resources overlap [79]. Based on experimental studies [82-84], the spacing between diet and bone apatite has been estimated to $10.7\% \pm 1.4\%$ on average (mean value) and between diet and enamel apatite to ca. 13.4‰ \pm 1.0‰ on average for a C₃ and a C₃-C₄ diet [85]. Hence, through the carbon apatite–collagen spacing ($\Delta^{13}C_{ap-coll}$) it is possible to distinguish the contribution of low (wide spacing) and high (small spacing) trophic level resources [86-88].

Materials

Archaeological wild and domestic terrestrial and aquatic animals, as well as archaeobotanical specimens were sampled from the same areas where the humans lived and shown in Fig 1.

There are 41 humans in total: 13 from La Barmaz, 12 from Vufflens and 16 from Tolochenaz. Sex and age-at-death were analyzed previously (Table 1) [30, 31, 35]. Sex was determined using standards outlined for the cranium and the coxal bones [89–91]. Age-at-death was assessed using criteria proposed by [92, 93] for adults and by [77, 94–96] for juveniles. To limit the uncertainties linked with using different methods, individuals were grouped into four age categories (Table 1). Collagen was extracted from cortical bone and dentine from the crown of the second molar, except for five individuals for whom samples were taken from the crown of a canine, a premolar, a first molar and two incisors.

Sites	ID	Burial	Grave goods	Skeletal element	Tooth	Sex	Age	Chronological period ^b	Date cal BCE (20)	Date BP
Collombey-Muraz, La Barmaz	BA3	N3	Р	ulna R	M ₂ R	F	A	EBA (BzA2)		
	BA5	N5	A	ulna L	M ₂ R	М	A	EBA		
	BA6a/b	N6a/N6b	nd	ulna L	M ₂ R	F	A	EBA		
	BA6	N6	Р	ulna L	M ₂ R	F	A	EBA (BzA2b,c)		
	BA22	N22	Р	ulna L	M ₂ R	М	A	EBA (BzA2)		
	BA23	N23	A	tibia R	M ₂ R	М	A	EBA		
	BA25	N25	A	ulna L	M ₂ R	М	A	EBA		
	BA26	N26	A	ulna L	M ₂ R	М	A	EBA		
	BA28	N28	A	humerus R	M ² R	F	A	EBA		
	BA42	N42	Р	ulna L	M ₂ R	М	YA	EBA (BzA2b)		
	BA50	T50	A	ulna L	M ₂ R	F	A	EBA	1618-1224 cal BCE	CRG 1339: 3172±82 BP
	BA53	N53	A	tibia R	-	М	A	EBA		
	ZHB	ZHB	Р	ulna L	M ₂ R	М	A	EBA (BzA2a)		
Vufflens-la-Ville, En Sency	VF1	VF 94/st 1 ind 1	Р	ulna R	M ₂ R	F	A	MBA (recent BzB)		
	VF2	VF 94/st 1 ind 2	Р	fibula	M ₂ R	М	YA	MBA (recent BzB)	1690-1430 cal BCE	ETH-15757: 3285±65 BP
	VF3	VF 95/st 4 ^b ind 1	Р	humerus R	M ₂ L	F	A	MBA (BzC)		
	VF4	VF 95/st 4 ^b ind 2	P?	humerus L	M ₂ R	I	С	MBA (BzC)	1690-1370 cal BCE	ETH-17761: 3235±80 BP
	VF5	VF 95/st 4 ^b ind 3	P?	ulna R	M ² L	F	YA	MBA (BzC)		
	VF6	VF 95/st 4 ^b ind 4	nd	ulna L	C sup R	I	С	MBA (BzC)		
	VF7	VF 95/st 4 ^b ind 5	Р	ulna R	M ₂ R	F	a	MBA (BzC)		
	VF8	VF 95/st 4 ^b ind 6	Р	ulna L	M ₂ L	F	A	MBA (BzC)	1520-1250 cal BCE	ETH-15758: 3120 ± 60 BP
	VF9	VF 95/st 9	Р	radius L	M ₂ L	М	A	MBA (BzB/C)	1530-1190 cal BCE	ETH-17758: 3130 ± 75 BP
	VF10	VF 95/st 10	Р	radius	M ₂ L	I	C	MBA (BzB/C)	1520-1260 cal BCE	ETH-15759: 3125 ± 55 BP
	VF11	VF 95/st 11	A	radius L	M2 R	М	a	MBA	1880-1500 cal BCE	ETH-17759: 3370 ± 70 BP
	VF12	VF 95/st 14	A	ulna R	M ₂ L	М	YA	MBA	1880-1510 cal BCE	ETH-17760: 3380 ± 70 BP
Tolochenaz, Le Boiron, La Caroline	LaC1	st. 1052	Р	radius R	M2 L	М	A	FBA (HaB3)		
	LaC2	st. 1061	Р	radius R	M2 R	F	A	FBA (early HaB1)		
	LaC3	st. 1074	Р	tibia R	M1 L	I	A	FBA (HaB2)		
	LaC4	st. 1111	Р	ulna L	PM1 L	M (?)	A	FBA (recent HaB3)		
	LaC5	st. 1057	A	ulna R	M2 R	I	C	FBA (HaB)		
	LaC6	st. 1080	Р	humerus	I2 R	I	C	FBA (HaB3)		
	LaC7	st. 1018	Р	clavicle R	I2 R	I	C	FBA (HaB1*)		
	LaC8	st. 1070	Р	femur L	-	I	C	FBA (HaB)		
	LaC9	st. 1083	Р	tibia R	-	I	A	FBA (HaB3)		
	LaC10	st. 1071	Р	femur R	-	I	A	FBA (HaB3)		
	LeB1	tb XXIV	nd	femur L	M2 L			FBA (?)		
	LeB2	tb IX-X	Р	femur R	-	М		FBA (early HaB3)		
	LeB3		nd	femur R	-			FBA		
	LeB4	tb I	nd	femur R	M2 R			FBA		
	LeB5	tb III	Р	femur L	M2 L			FBA (recent HaB3)		
	LeB6		nd	humerus L	M2 R			FBA		

Table 1. Human remains analyzed with their relevant anthropological and archaeological information.

^aThe chronology is according to [13, 97, 98].

^bVF 95/st 4: presence of grave goods that could not be attributed to a specific individual.

Abbreviations: R = right; L = left; child (C) = 5–15 yrs; adolescent (a) = 16–19 yrs; young adult (YA) = 20–29 yrs; adult (A) > 30 yrs; M = male; F = female; I = indeterminate; EBA = Early Bronze Age; MBA = Middle Bronze Age; FBA = Final Bronze Age; Grave goods: P = present, A = absent, ND = not defined.

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The terrestrial animals date to the Early (La Barmaz), Middle (Rances, Champ Vully) and Final Bronze Age (Chens-sur-Léman, Tougues). They include domestic and wild animals (*Ovis aries, Capra hircus, Bos Taurus, Sus domesticus, Cervus elaphus*) for a total of 30 individuals. Four samples of pike (*Esox lucius*) were sampled from Chindrieux (Lake of Bourget, Savoie), dating to the Final Bronze Age (Table A in S1 Text).

In addition, 30 seeds and chaff from cultivated plants from Chens-sur-Léman (Pré d'Ancy and Vereître) were analyzed (Table A in <u>S1 Text</u>). They are dated to the Early, Middle and

Final Bronze Age in order to highlight any possible changes in agricultural practices across time. The C_3 plant samples include hulled barley (*Hordeum vulgare*), emmer and einkorn wheat (*Triticum dicoccum*, *Triticum monococcum*), and pulses, such as beans (*Vicia faba*), for a total of 25 samples. The C_4 plant samples include five seeds of broomcorn and foxtail millet (*Panicum miliaceum*, *Setaria italica*). Each isotopic measurement corresponds to the analysis of one seed.

Methods

La Barmaz humans are in storage at the University of Geneva and animals are in the deposit of the Office for Archaeological Research of Valais; sampling permits were issued by the Office for Archaeological Research of Valais. Vufflens-la-Ville humans are stored in the Cantonal Museum of Archaeology and History of Lausanne and Tolochenaz humans in the store of Archeodunum SA and sampling permits were granted by the Cantonal Museum of Archaeology and History of Lausanne. Animals from Tougues and Chindrieux are stored in the deposit of the DRAC Auvergne-Rhône-Alpes Regional Archaeology Service and Rances animals are in the Natural History Museum of Geneva and the DRAC Auvergne-Rhône-Alpes provided the sampling authorizations. The botanical remains are stored in the Archaeological Research Center of Clermont-Ferrand and study permission was provided by the National Institute for Preventive Archaeological Research (INRAP).

Samples were prepared at the LAMPEA (UMR 7269, Aix-en-Provence, France) and the EA-IRMS analyses were performed at the Iso-Analytical Limited Laboratory (Cheshire, UK). The seeds and chaff were analyzed following the acid-base-acid protocol [69]. The samples were soaked in HCl (0.5 M, 70°C, for 30–60 min), then rinsed three times with distilled water and soaked in NaOH (0.1 M, 70°C, 60 min) and rinsed again and soaked in HCl (0.5 M, 70°C, for 30–60 min). Once dried in the oven, the samples were finely powdered.

Bone and dentine collagen was extracted following [99] and modified by [100]. Approximately 0.5 g of cortical bone was cleaned by surface abrasion to remove superficial contaminants and demineralized (0.5 M HCl, 4°C, for 7–14 days). The samples were then rinsed several times with distilled water and gelatinized (pH 3, 75°C, for 48 h). The resulting solution was filtered using an Ezee[™] filter and the supernatant was freeze-dried for 48 h. Similarly, for each selected tooth, half the crown was cut with a saw and the samples were demineralized (0.5 M HCl, 4°C, for ca. 20 days). Once the dentine was soft, the samples were rinsed with distilled water and gelatinized (pH 3, 75°C, for 48 h). The solution was then filtered with Ezee[™] filter and the supernatant was freeze-dried for 48 h, as was done for the bone.

Tooth enamel carbonates was pre-treated following the protocol of [101] on enamel powder. The samples were placed in NaOCl (2%–3%, 0.1 ml/1 mg, 24 h), rinsed and soaked in CH3COOH to remove diagenetic carbonates (1 M, 1 h, 1 mL/1 mg), rinsed again in a centrifuge and dried (65°C, 12 h).

No consensual criteria exist to assess the preservation of charred seeds and chaff and whether or not the resultant values are reliable. Here, the criteria proposed by [102] for %C, % N and C:N ratio are considered. Taking into account the average offsets between uncarbonized crop seeds and those heated for four, eight or 24 h at 215, 230, 245 or 260°C, the δ^{13} C and δ^{15} N values of carbonized crop were corrected for the charring effect by subtracting 0.11‰ and 0.31‰, respectively, according to the recommendations of [103]. Concerning the three chaff samples of *Triticum*, no criteria to establish the reliability of the results exist. Following the suggestions of two experimental studies, considering the offset between cereal grain and rachis [70, 104], +2.4‰ in δ^{15} N and +1.9‰ in δ^{13} C were added to the wheat chaff values in order to make the seed and the chaff isotope results comparable.

Bone and dentine collagen preservation was checked according to the following criteria: the yield of extraction ($\geq 1\%$), the percentages of C, N and S (%C $\geq 30\%$, %N $\geq 10\%$ and % S $\geq 0.15\%$), and the atomic C:N, C:S and N:S ratio (2.9 < C:N < 3.6, C:S = 600 ± 300 and N: S = 200 ± 100) [105–108]. For the tooth enamel carbonates, no set rules exist to distinguish reliable apatite samples [109, 110] because the apatite carbonate preservation in archaeological samples is difficult to estimate [111]. Even though enamel can be subject to alteration in certain burial conditions [111, 112], enamel is less porous than bone, limiting the risk of diagenetic modifications induced by contamination and degradation [111, 113].

The international standards used for collagen and bulk plants are bovine liver (IA-R042), a mixture of ammonium sulfate (IA-R045) and beet sugar (IA-R005), and a mixture of sugar cane (IA-R006) and ammonium sulfate (IA-R046). For carbonate apatite, they are calcium carbonate (IA-R022), carbonatite (NBS-18), chalk (IA-R066), and Carrara marble (IAEA-CO-1). Measurement reproducibility of a repeat sample is below 0.1‰ for $\delta^{13}C_{coll}$, $\delta^{13}C_{enamel}$, $\delta^{15}N$ and $\delta^{34}S$ values.

To reconstruct the dietary scenarios, two offsets were used for carbon: (a) 4.8‰ between the δ^{13} C values of the plants and the collagen of their consumers and (b) 0.8‰ between the carbon isotope values of the collagen of consumers of the previous trophic level [114]. For nitrogen isotope compositions, a spacing of 4.0‰ was considered between the diet and the consumer of two different trophic levels [114].

For data interpretation and statistical analyses, Microsoft Excel and R v.3.6.1 were used. Bayesian models were elaborated using the software FRUITS [115].

Results

Botanical data

Of the 30 botanical remains, only one barley seed (VR6) was excluded after pre-treatment because it did not have enough material for analysis. The %C, %N and the C:N atomic ratios for all the other charred seeds fit within the expected ranges [102], with the exception of the % C of VE9 which is lower than in the others (17.7%) (Table 2).

The isotopic ratios of the C₃ plants range from 1.1‰ to 6.7‰ for δ^{15} N (3.6‰ ± 1.5‰, n = 24) and from 27.0‰ to -15.3‰ for δ^{13} C (-24.3‰ ± 2.2‰, n = 24). The C₄ samples results range from 5.3‰ to 6.4‰ for δ^{15} N (5.7‰ ± 0.5‰, n = 5) and from -10.8‰ to -9.4‰ for δ^{13} C (-10.3‰ ± 0.5‰, n = 5). When pooling together all the samples of the same species in each of the different phases, no significant differences in N among species are detected, possibly due to the small sample size (approximate Kruskal-Wallis *p* = 0.103). In fact, the δ^{15} N value of millet appears higher when compared to the other species. Significant differences by species are highlighted for δ^{13} C (*p* = 0.001). When considering only C₃ plants, no significant differences appear (*p* = 0.498) (Fig 2, Table 3).

Animal and human data

Of the 22 animal samples, 19 have a good yield. Collagen preservation and carbon, nitrogen and sulfur are present in enough quantity attesting to the reliability of the results. Two cattle (BANF6 and BANF7) with %N, %C and %S below the accepted values but very close to the accepted limits, have been included in the study but are considered with caution. Similarly, a pig (TO9) showing C:S and N:S slightly below the accepted criteria has been included in the study and is also evaluated with caution (Table 4). A pig, a sheep/goat and a pike (BANF2, BANF3, CHIN4) have been excluded because the %N, %C, %S, C:S and N:S do not fall within the acceptable range (Table 4).

Id	Chronological period	Species	Sample	%N	$\delta^{15}N_{AIR}$	%C	$\delta^{13}C_{V-PDB}$	C/N	$\Delta^{13}C^a$
PAbot1	MBA-FBA (MB II-FB I)	Vicia faba	seed	4.7	2.6	44.9	-23.1	11.2	17.0
PAbot2	MBA-FBA (MB II-FB I)	Hordeum vulgare	seed	2.5	3.0	57.8	-24.8	27.2	18.8
PAbot3	FBA (FB IIb-IIIa)	Panicum miliaceum	seed	3.6	5.5	51.7	-9.4	16.9	
PAbot4	MBA-FBA (BM II-FB I)	Hordeum vulgare	seed	2.0	3.6	61.0	-24.9	35.1	18.9
PAbot5	MBA-FBA (BM II-FB I)	Hordeum vulgare	seed	2.0	4.0	55.6	-24.2	31.9	18.2
PAbot6	MBA-FBA (MB II-FB I)	Fabaceae	seed	4.5	3.3	50.6	-15.3	13.1	9.0
PAbot7	MBA-FBA (MB II-FB I)	Fabaceae	seed	7.7	1.8	56.3	-23.9	8.5	17.9
PAbot8	FBA (FB IIb/IIIa)	Fabaceae	seed	6.3	1.1	53.7	-24.2	10.0	18.2
PAbot9	EBA	<i>Triticum</i> sp	seed	3.0	4.4	60.1	-27.0	23.5	21.2
PAbot10	EBA	<i>Triticum</i> sp	seed	3.0	3.7	60.6	-26.2	23.3	20.3
PAbot11	EBA	<i>Triticum</i> sp	seed	1.9	3.6	53.8	-23.9	32.4	18.0
VE1	FBA (FB IIb)	Triticum dicoccum	seed	1.7	2.1	48.7	-24.7	33.3	18.7
VE2	FBA (FB IIb)	Triticum dicoccum	seed	2.8	3.4	43.9	-23.6	18.5	17.5
VE3	FBA (FB IIa)	Triticum dicoccum	seed	1.9	6.3	34.4	-23.6	21.5	17.5
VE4	FBA (FB IIa)	Triticum dicoccum	seed	3.0	3.1	54.7	-24.4	21.5	18.4
VE5	FBA (Early Ha)	Hordeum vulgare	seed	6.1	2.2	62.1	-24.3	12.0	18.2
VE6	FBA (Early Ha)	Hordeum vulgare	seed		Not	enough ma	aterial to be anal	ysed	
VE7	FBA (FB IIa)	Panicum miliaceum	seed	3.0	6.1	52.7	-10.8	20.4	
VE8	FBA (FB IIa)	Vicia sp	seed	5.6	2.8	61.6	-26.1	12.9	20.2
VE9	FBA (FB IIa)	Setaria italica	seed	1.1	6.4	17.7	-10.5	19.2	
VE10	FBA (Early Ha)	Vicia sp	seed	3.1	6.7	52.1	-24.1	19.6	18.0
VE11	FBA (Early Ha)	Vicia sp	seed	1.6	6.5	46.4	-24.5	34.0	18.5
VE12	FBA (Early Ha)	<i>Vicia</i> sp	seed	2.0	5.3	64.6	-26.5	37.1	20.5
VE13	FBA (FB IIa)	Triticum monococcum/dicoccum	chaff	0.8	4.6	62.1	-25.4	87.4	17.4
VE14	FBA (Early Ha)	Triticum monococcum/dicoccum	chaff	0.7	2.5	59.4	-25.8	100.4	17.7
VE15	FBA (FB IIa)	Setaria/Panicum	seed	3.0	5.4	61.0	-10.1	23.9	
VE16	FBA (FB IIa)	Setaria/Panicum	seed	2.7	5.3	61.8	-10.5	26.3	
VE17	FBA (FB IIa)	<i>Triticum</i> sp	seed	2.1	2.6	54.0	-23.5	29.4	17.4
VE18	FBA (FB IIa)	<i>Triticum</i> sp	seed	2.6	5.7	53.1	-24.5	24.1	18.4
VE19	FBA (FB IIa)	<i>Triticum</i> sp	chaff	0.5	2.7	60.1	-25.3	133.3	17.2

Table 2. Isotopic results of the archaeolog	gical plant rema	ins from Chens-sur-Léma	n, Pré d'Ancy (PA	A) and Vereître (VE).
				-,	,.

^aCalculated on the corrected values (-0.11‰ to δ^{13} CV-PDB of the charred seeds and +1.9‰ to δ^{13} CV-PDB of the chaff) using AIRCO2_LOESS data calibrator [71].

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The terrestrial animals show δ^{15} N values between 2.7‰ to 8.7‰ (5.8‰ ± 1.6‰, n = 16), between -22.7‰ to -20.0‰ for δ^{13} C (-21.1‰ ± 0.5‰, n = 16) and between 0.7‰ and 8.7‰ for δ^{34} S (4.2‰ ± 2.1‰, n = 15). Two of the pigs, RCV10 and TO7, have high nitrogen values suggesting an important animal protein intake, whereas all the other pigs present typical herbivore values.

A Kruskal-Wallis test does not highlight differences for δ^{13} C and δ^{15} N between the Early, Middle and Final Bronze Age (p = 0.758 and p = 0.102, respectively), conversely to δ^{34} S which displays significant differences (p = 0.027) (Table 5). An Exact Wilcoxon Mann Whitney test between the Early and Final Bronze Age animals does not show any significant differences (p = 0.212). An Exact Wilcoxon Mann Whitney test for the Middle Bronze Age animals was not run because only two samples are available. However, it is evident that these individuals have higher sulfur values than all the other animals.

The three pike range from 9.8‰ to 10.7‰ for $\delta^{15}N$ (10.3‰ ± 0.5‰, n = 3), between -23.8‰ and -21.5‰ for $\delta^{13}C$ (-22.9‰ ± 1.2‰, n = 3) and between -5.7‰ and -1.8‰ for $\delta^{34}S$



Fig 2. Box-plots showing the stable isotope results for wheat chaff and charred seeds. Corrected values have been used for a) δ^{15} N results (-0.31‰ for the grains and +2.4‰ for the chaff to eliminate the charring effect) and b) δ^{13} C results (-0.11‰ for the grains and +1.9‰ for the chaff). The boxes represent the first and third quartiles and the whiskers the maximum and minimum values, excluding the outliers. The continuous line illustrates the median and the cross represents the mean. For δ^{13} C results only C₃ plants are represented.

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 $(-4.3\% \pm 2.1\%, n = 3)$. The negative sulfur values are not surprising considering that the δ^{34} S of sulfates from riverine ecosystems mainly range from -5 to +15‰, with outliers resulting from geochemical processes of sulfate reduction or sulfide oxidation [61, 116, 117].

Bone collagen was extracted from 41 humans. All the results obtained from La Barmaz and Vufflens respect the quality criteria; three samples from Tolochenaz, LaC1, LaC4 and LaC10, were excluded because the %N and %C values do not respect the accepted ranges. Due to dating problems, LeB1 is excluded from the interpretations.

The δ^{15} N values range between 7.2‰ and 10.1‰ (8.6‰ ± 0.8‰, n = 37), δ^{13} C between -21.2‰ and -17.6‰ (-19.8‰ ± 1.2‰, n = 37) and δ^{34} S between -2.6‰ and 8.9‰ (2.9‰ ± 2.1‰, n = 36) (Table 6).

When pooling together all the individuals from the three sites, significant differences appear for δ^{15} N (p < 0.001), δ^{13} C (p < 0.001) and δ^{34} S (p = 0.002) (Table 7). An Exact Wilcoxon test between pairs of sites (Table B in <u>S1 Text</u>) shows significant differences for δ^{13} C values for Tolochenaz when compared to Vufflens (p < 0.001) and La Barmaz (p < 0.001). For δ^{15} N,

		-								
		$\delta^{15}N_A$	a AIR						$\delta^{13}C_{V-PDB}^{b}$	
species	n	min	max	Mean (1sd)	p (Kruskas Wallis)	min	max	Mean (1sd)	p (Kruskas Wallis)	p (Kruskas Wallis)
wheat	12	1.8	7.0	4.7 (1.6)	0.103	-27.1	-23.4	-24.4 (1.2)	0.001	0.498
barley	4	1.9	3.7	2.9 (0.8)		-24.9	-24.2	-24.7 (0.4)		
pulses	8	0.8	6.4	3.5 (2.1)		-26.5	-15.3	-23.6 (3.5)		
millet	4	5.0	6.1	5.4 (0.5)		-10.9	-9.6	-10.4 (0.5)		

Table 3. Statistical parameters for the botanical remains.

 a Correction of -0.31‰ for $\delta^{15}N_{AIR}$ to eliminate the effect of carbonization and +2.4‰ for chaff.

 b Correction of -0.11‰ to $\delta^{13}C_{V\text{-PDB}}$ for the charred seeds and +1.9‰ for the chaff.

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ID	Chronological period	Species/family	Skeletal element	N (%)	$\delta^{15}N_{AIR} (\%)$	C (%)	δ ¹³ C _{V-PDB} (‰)	S (%)	δ ³⁴ S _{V-CDT} (‰)	C:N	C:S	N:S
BANF1	EBA	Sus domesticus	humerus	13.7	5.8	37.7	-21.0	-	-	3.2	-	-
BANF2	EBA	Ovis aries/Capra hircus	tibia	3.3	5.3	9.5	-21.2	0.06	1.8	3.4	399.0	117.4
BANF3	EBA	Sus domesticus	humerus	0.1	-	1.5	-	0.05	2.9	18.6	88.3	4.8
BANF5	EBA	Ovis aries/Capra hircus	tibia	14.6	5.3	39.7	-21.0	0.20	4.3	3.2	532.2	168.5
BANF6	EBA	Bos taurus	talus	10.3	4.8	28.0	-21.0	0.13	3.5	3.2	565.8	179.5
BANF7	EBA	Bos taurus	talus	9.9	4.7	27.3	-21.2	0.12	3.8	3.2	588.4	182.8
BANF8	EBA	Bos taurus	talus	14.9	3.9	40.6	-21.1	0.20	5.2	3.2	548.2	173.2
RCV4	MBA	Bos taurus	humerus	14.3	5.9	39.2	-21.4	0.20	8.4	3.2	403.3	127.1
RCV10	MBA	Sus domesticus	mandible	12.7	8.7	34.9	-20.0	0.18	8.7	3.2	431.1	136.2
TO1	FBA	Ovis aries	radius	15.1	6.5	41.1	-21.6	0.27	3.1	3.2	389.9	122.3
TO2	FBA	Bos taurus	radius	14.7	6.6	40.1	-21.4	0.25	4.7	3.2	339.0	106.2
TO4	FBA	Cervus elaphus	radius	15.2	5.9	41.7	-21.3	0.28	1.8	3.2	373.4	116.5
TO5	FBA	Capra hircus	ulna	14.2	3.7	39.1	-21.4	0.31	3.7	3.2	331.1	102.1
TO7	FBA	Sus domesticus	humerus	14.9	8.5	41.0	-20.3	0.29	3.5	3.2	298.5	91.7
TO8	FBA	Sus domesticus	humerus	15.0	6.4	41.8	-20.4	0.33	4.4	3.3	386.2	121.1
TO9	FBA	Sus domesticus	mandible	14.2	6.3	39.6	-21.0	0.35	3.2	3.3	335.2	104.5
TO11	FBA	Bos taurus	humerus	14.6	2.7	40.0	-22.0	0.27	0.7	3.2	403.3	127.1
TO13	FBA	Ovis aries	tibia	14.2	7.0	39.2	-20.7	0.31	4.2	3.2	431.1	136.2
CHIN1	FBA	Esox lucius	vertebra	14.9	10.7	45.3	-21.5	0.60	-1.8	3.5	199.1	56.4
CHIN2	FBA	Esox lucius	vertebra	14.5	9.8	44.2	-23.4	0.55	-5.7	3.5	213.0	60.3
CHIN3	FBA	Esox lucius	vertebra	15.1	10.4	45.3	-23.8	0.61	-5.2	3.5	198.2	57.0
CHIN4	FBA	Esox lucius	hyomandibular	1.5	10.3	7.0	-24.1	0.21	-6.7	5.4	89.2	16.5

Table 4.	δ¹	⁵ Ν, δ	¹³ C and	lδ ³⁴ 9	5 values	of an	nimal	bone	collagen.
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Sites: Collombey-Muraz, La Barmaz (BANF); Rances, Champ Vully (RCV); Chindrieux (CHIN); Chens-sur-Léman, Tougues (TO).

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slight differences are evident between La Barmaz and Vufflens (p = 0.040) and between Vufflens and Tolochenaz (p < 0.001). For δ^{34} S, differences are present between La Barmaz and Vufflens (p = 0.009) and between La Barmaz and Tolochenaz (p = 0.004). Differences within each community have also been tested according to biological parameters. Given the number of individuals, statistical analyses were performed only on La Barmaz and Vufflens when considering sex differences. No statistically significant differences appear within these communities (Table C in S1 Text). Concerning age-at-death, statistical analysis was only possible for Vufflens and no statistically significant differences have been detected (Table C in S1 Text). When considering presence-absence of grave goods, no differences are highlighted for La Barmaz (δ^{13} C, p = 0.508; δ^{15} N, p = 0.086; δ^{34} S, p = 0.422). For Vufflens and Tolochenaz, given the difficulties in attributing specific grave goods to some individuals, statistical analyses were not performed for this parameter.

Dentine collagen was extracted from 35 individuals and all the samples respect the quality conditions. The δ^{15} N values range between 7.3‰ and 10.4‰ (9.1‰ ± 0.9‰, n = 34), for δ^{13} C between -21.5‰ and -16.4‰ (-19.8‰ ± 1.5‰, n = 34). Significant differences occur for δ^{13} C (p < 0.001) (Table 7). An Exact Wilcoxon test highlights differences for δ^{13} C between La Barmaz and Tolochenaz (p < 0.001) and between Vufflens and Tolochenaz (p < 0.001) (Table B in S1 Text). Like for the bone collagen results, Tolochenaz δ^{13} C values are more enriched in 13 C compared to the other two sites. The test highlights no differences in δ^{15} N values among

	δ ¹⁵ N _{AIR}								$\delta^{13}C_{V-PD}$	В	$\delta^{34}S_{V-CDT}$			т
	species	n	min	max	Mean (1sd)	p (Kruskas wallis)	min	max	mean (1sd)	p (Kruskas wallis)	min	max	mean (1sd)	p (Kruskas wallis)
Collombey-Muraz, La	Pig	1	5.8	5.8	-	0.102	-21.0	-21.0	-	0.758	-	-	-	0.027
Barmaz Early Bronze Age	Sheep/ goat	1	5.3	5.3	-		-21.0	-21.0	-		4.3	4.3	-	
	cattle	3	3.9	4.8	4.5 (0.5)		-21.2	-21.0	-21.1 (0.1)	_	3.5	5.2	4.2 (0.9)	
	ТОТ	5	3.9	5.8	4.9 (0.7)		-21.2	-21.0	-21.1 (0.1)	_	3.5	5.2	4.2 (0.8)	
Rances, Champ Vully Middle	pig	1	8.7	8.7	-		-20.0	-20.0	-		8.7	8.7	-	
Bronze Age	cattle	1	5.9	5.9	-		-21.4	-21.4	-		8.4	8.4	-	
	ТОТ	2	5.9	8.7	7.3 (2.0)		-21.4	-20.0	-20.7 (1.0)		8.4	8.7	8.6 (0.2)	
Chens sur Léman Final Bronze Age	pig	3	6.3	8.5	7.1 (1.3)		-21.0	-20.3	-20.6 (0.4)		3.2	4.4	3.7 (0.6)	
	Sheep/ goat	3	3.7	7.0	5.7 (1.8)		-21.6	-20.7	-21.2 (0.4)		3.1	4.2	3.7 (0.6)	
	cattle	2	2.7	6.7	4.6 (2.8)		-22.1	-21.4	-21.7 (0.5)		0.7	4.8	2.7 (2.9)	
	Red deer	1	5.9	5.9	-		-21.3	-21.3	-		1.8	1.8	-	
	ТОТ	9	2.7	8.5	5.9 (1.7)		-22.1	-20.3	-21.1 (0.4)	<u> </u>	0.7	4.8	3.3 (1.3)	
	pike	3	9.8	10.7	10.3 (0.5)		-23.8	-21.5	-22.9 (1.2)		-5.7	-1.8	-4.3 (2.1)	

Table 5. Statistical parameters for the animal bone collagen.

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the three communities. In terms of biological criteria, differences by sex are non-significant. With regards to age-at-death, statistical analysis is only possible for Vufflens and no differences are detected (Table C in <u>S1 Text</u>). No differences are present in La Barmaz when results are analyzed by presence-absence of grave goods (δ^{13} C, *p* = 0.158; δ^{15} N, *p* = 0.222).

The presence of significant differences between the bone and the teeth values of each individual within each human group were tested. The Exact Wilcoxon test for dependent samples highlights differences in La Barmaz for the δ^{15} N values, as teeth values are higher than those from bone (p = 0.024). In Vufflens and Tolochenaz differences are evident for δ^{13} C values. In the Vufflens individuals, bone has higher δ^{13} C values than teeth (p = 0.019), whereas in Tolochenaz the opposite trend is observable, teeth being enriched in 13 C as opposed to bone (p = 0.046) (Table 8).

For the tooth enamel carbonates, 34 individuals were analyzed for $\delta^{13}C_{enamel.}$ Values range from -15.4‰ to -9.1‰ (-12.9 ± 1.5‰, n = 34). Significant differences are present among the three sites (Exact Kruskal Wallis test, p < 0.001) (Tables 6 and 7, S4 Fig). A Wilcoxon test comparing the sites by pairs reveals significant differences (Table B in S1 Text). The three groups clearly show different patterns indicating specific diets. Like for the bone and the dentine collagen, Tolochenaz displays higher δ^{13} C values compared to the other two groups. Vufflens compared to La Barmaz also shows slightly higher δ^{13} C values than La Barmaz. No significant differences are observable between La Barmaz and Vufflens according to sex and age-at-death (Table C in S1 Text).

Discussion

Soil management and farming strategies

The wide δ^{15} N plant range (Δ^{15} N = 5.9) reveals an important variability in nitrogen isotopic values. The use of manure to improve soil fertility leads to an enrichment in ¹⁵N of cultivated

	CO2 (ml)	0.056	0.036	0.046	0.042	0.030	0.040	0.035	0.041	0.029	0.036	0.037	I	0.040	0.020	0.031	0.033	0.037	0.036	0.045	0.027	0.028	0.042	
(leme	Sample weight used (mo)	6.07	5.91	5.71	5.95	5.32	5.91	6.10	6.04	4.44	4.89	6.17	I	6.02	6.15	6.07	5.25	6.04	6.14	6.01	5.24	5.69	5.52	ç
A D A TITE (on	δ ¹³ C _{enamel} (%0)	-14.1	-15.1	-13.8	-14.3	-15.4	-14.5	-14.3	-13.7	-15.0	-14.1	-12.9	I	-14.8	-13.7	-13.7	-13.7	-13.1	-13.7	-13.0	-13.4	-13.6	-12.8	
	Carbonate content	4.16	2.73	3.59	3.16	2.50	3.04	2.54	3.01	2.87	3.29	2.70	I	2.98	1.47	2.29	2.82	2.77	2.63	3.33	2.30	2.23	3.36	
(au	8 ¹³ C (%o)	-20.1	-20.4	-20.1	-20.7	-20.8	-20.4	-20.8	-20.3	-21.1	-21.1	-20.0	,	-21.1	-20.8	-20.6	-20.6	-20.5	-21.5	-20.5	-21.0	-20.7	-20.5	
I (dant	C (%)	42.8	45.2	45.4	42.1	43.2	43.5	43.5	44.7	45.7	45.7	46.5	1	44.5	44.6	44.3	44.7	34.1	41.1	43.4	43.0	44.0	35.9	
LACEN	8 ¹⁵ N (%0)	9.1	8.6	8.6	8.9	9.6	11.8	8.8	10.0	7.9	8.5	9.8	1	8.1	8.8	8.8	10.1	9.5	10.3	10.2	8.7	8.8	10.4	
100	Z (%)	15.9	16.4	16.9	15.0	16.1	16.0	16.0	16.6	17.0	16.6	16.2	1	16.2	16.5	16.5	16.6	12.3	14.4	16.0	15.3	16.1	12.8	
	N/S	153.0	181.1	150.3	183.9	148.6	161.5	135.4	148.2	139.4	151.7	142.6	136.2	154.6	137.9	143.3	118.4	131.0	135.6	122.2	126.6	120.3	111.3	
	C/S	485.2	570.4	488.5	580.6	510.0	554.8	470.2	514.6	484.8	526.2	493.7	475.1	505.6	479.4	505.1	410.1	457.6	477.0	445.6	442.9	422.2	393.6	
	δ ³⁴ S (%o)	3.5	-2.6	2.0	1.8	0.8	1.6	1.0	-0.8	2.3	0.8	4.2	0.8	4.1	3.7	3.1	4.4	3.8	2.0	3.6	2.1	3.7	2.6	
(anot	%S (%)	0.21	0.19	0.19	0.18	0.23	0.23	0.26	0.24	0.26	0.25	0.22	0.26	0.19	0.25	0.22	0.28	0.25	0.24	0.25	0.26	0.27	0.27	
CEN (1	νC	3.2	3.2	3.3	3.2	3.4	3.4	3.5	3.5	3.5	3.5	3.5	3.5	3.3	3.5	3.5	3.5	3.5	3.5	3.7	3.5	3.5	3.5	
enamel-	8 13 Ccoll (%0)	-20.5	-20.4	-20.1	-20.4	-20.8	-20.5	-20.8	-20.5	-20.9	-21.2	-20.2	-20.9	-20.6	-20.4	-20.4	-20.3	-20.3	-20.9	-20.9	-20.5	-20.5	-20.5	
nd & ¹³ C	C (%)	38.5	40.9	35.9	40.4	44.6	47.2	45.2	46.1	47.7	49.7	41.8	46.6	37.0	44.5	41.8	43.3	43.8	42.9	42.1	43.0	42.9	39.7	
lagen a	§ ¹⁵ N (%0)	8.3	8.4	7.8	8.4	8.8	10.1	8.9	10.0	8.0	7.7	9.0	8.2	8.3	0.6	9.6	9.5	8.8	10.0	9.5	8.6	8.7	9.8	
ine col	N (%)	14.1	15.1	12.8	14.9	15.1	16.0	15.1	15.4	15.9	16.7	14.0	15.5	13.1	14.9	13.8	14.5	14.6	14.2	13.4	14.3	14.2	13.0	
l denti	Age	A	A	¥	A	A	A	A	A	A	ΥA	A	A	A	V	YA	V	υ	YA	υ	63	V	A	
ne and	Sex	щ	М	ц	н	Μ	Μ	М	Μ	ц	М	Ь	М	Я	ц	M	щ	ц	щ	н	щ	ц	М	
ıman bo	Tooth	$M_2 R$	$M_2 R$	$M_2 R$	$M_2 R$	$M_2 R$	$M_2 R$	$M_2 R$	$M_2 R$	$M^2 R$	$M_2 R$	$M_2 R$	ı	$M_2 R$	$M_2 R$	$M_2 R$	$M_2 L$	$M_2 R$	$M^{2}L$	C sup R	$M_2 R$	$M_2 L$	$M_2 L$	
δ ¹³ C _{col} of hι	Skeletal element	ulna R	ulna L	ulna L	ulna L	ulna L	tibia R	ulna L	ulna L	humerus R	ulna L	ulna L	tibia R	ulna L	ulna R	fibula	humerus R	humerus L	ulna R	ulna L	ulna R	ulna L	radius L	
δ ¹⁵ N and	Burial	N3	N5	N6a/ N6b	N6	N22	N23	N25	N26	N28	N42	T50	N53	ZHB	VF 94/ st 1 ind 1	VF 94/ st 1 ind 2	VF 95/ st 4 ind.1	VF 95/ st 4 ind 2	VF 95/ st 4 ind 3	VF 95/ st 4 ind 4	VF 95/ st 4 ind 5	VF 95/ st 4 ind 6	VF 95/ st 9	
Table 6.	Ð	BA3	BA5	BA6a/ b	BA6	BA22	BA23	BA25	BA26	BA28	BA42	BA50	BA53	ZHB	VF1	VF2	VF3	VF4	VF5	VF6	VF7	VF8	VF9	

										SEN 0	(onod				8	II AGE	V (dan	tina)		A D A TITE (an	(lent	
Ð	Burial	Skeletal	Tooth	Sex	Age	z	δ ¹⁵ N	C	δ ¹³ C _{coll}	C]	%S	8 ³⁴ S	C/S	N/S	Z	δ ¹⁵ Ν	C	δ ¹³ C	Carbonate	δ ¹³ C _{enamel}	Sample	C02
		element				(%)	(‰)	(%)	(%)	z	(%)	(‰)			(%)	(‰)	(%)	(‰)	content	(%)	weight used (mg)	(Iml)
VF10	VF 95/ st 10	radius	$M_2 L$	I	υ	14.7	8.8	44.4	-20.6	3.5	0.22	2.8	533.0	152.1	16.2	9.3	43.5	-20.8	1.75	-13.6	5.97	0.023
VF11	VF 95/ st 11	radius L	$M_2 R$	М	a	14.8	8.9	44.5	-20.4	3.5	0.24	2.9	483.1	138.1	15.6	9.7	42.7	-20.4	2.27	-13.9	6.08	0.031
VF12	VF 95/ st 14	ulna R	$M_2 L$	М	ΥA	14.7	8.4	44.0	-20.5	3.5	0.25	2.8	474.4	136.2	16.1	8.5	44.4	-21.2	3.38	-13.8	5.34	0.040
LaC1	st. 1052	radius R	$M_2 L$	Μ	A	10.2	8.6	35.8	-17.6	4.1	0.13	7.4	722.9	176.3	16.1	9.6	43.7	-20.3	2.39	-13.3	5.99	0.032
LaC2	st. 1061	radius R	$M_2 R$	н	A	14.8	7.3	44.5	-18.0	3.5	0.19	2.8	609.0	174.6	16.1	7.3	43.6	-18.3	2.57	-10.8	5.93	0.034
LaC3	st. 1074	tibia R	M^1L	п	Α	10.8	8.6	30.5	-18.5	3.3		,	,	,	15.5	8.5	42.1	-16.7	2.47	-9.3	5.59	0.031
LaC4	st. 1111	ulna L	PM ¹ L	M (5)	A	6.4	8.6	18.2	-18.2	3.3	0.12	6.7	410.8	124.2	16.4	8.4	44.1	-17.1	2.21	-9.1	6.08	0.030
LaC5	st. 1057	ulna R	$M^2 R$	I	C	12.9	7.5	34.8	-18.4	3.2	0.17	5.7	530.3	168.9	16.1	8.0	44.1	-18.3	4.15	-10.6	5.18	0.048
LaC6	st. 1080	humerus	$I_2 R$	Ι	U	11.4	7.7	31.1	-18.4	3.2	0.16	4.6	515.1	162.3	14.9	9.4	40.8	-17.8	3.22	-10.4	5.93	0.043
LaC7	st. 1018	clavicle R	$I_2 R$	п	υ	14.5	8.1	39.0	-18.3	3.1	0.19	4.0	542.4	173.4	15.1	9.9	40.7	-16.4	2.99	-9.4	5.61	0.038
LaC8	st. 1070	femur L	ı	г	υ	13.8	8.0	37.8	-19.1	3.2	0.18	5.0	545.6	170.8	'	'	'				,	,
LaC9	st. 1083	tibia R	ı	I	A	13.0	7.6	35.2	-17.6	3.2	0.16	4.1	594.1	188.2	'	'	'					,
LaC10	st. 1071	femur R	ı	г	A	1.0		3.9	ı	'	,				'	'	'					,
LeBI	tb XXIV	femur L	M_2L			14.6	9.2	44.1	-20.5	3.5	0.23	0.9	514.5	146.9	15.4	9.7	41.6	-20.3	1.46	-14.4	6.03	0.020
LeB2	tb IX-X	femur R	ı	М		10.4	8.3	31.8	-17.2	3.5	0.20	1.3	423.8	120.0	'	'	'					,
LeB3		femur R	ı			15.6	9.7	46.7	-20.4	3.5	0.20	2.9	604.4	173.7	'	'	'				,	,
LeB4	tb I	femur R	$M_2 R$			13.8	7.2	42.1	-18.2	3.6	0.22	1.2	505.7	142.6	13.8	7.5	38.9	-17.7	3.33	-10.1	5.42	0.040
LeB5	tb III	femur L	$M_2 L$			14.1	8.4	42.3	-17.2	3.5	0.20	8.9	556.4	159.8	14.9	10.1	41.2	-17.1	2.70	-9.9	5.92	0.036
LeB6		humerus L	$M_2 R$			15.6	8.6	47.0	-17.5	3.5	0.19	6.7	638.9	182.5	15.6	8.5	42.1	-17.4	1.53	-10.3	5.95	0.020
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Table 6. (Continued)

	δ ¹⁵ N	1			δ ¹³ C	coll			$\delta^{34}S$				$\delta^{13}C_{enamel}$			
Collombey-Muraz, La							Bone									
Barmaz	n	min	max	Mean (1sd)	n	min	max	Mean (1sd)	n	min	max	Mean (1sd)				
	13	7.7	10.1	8.6 (0.7)	13	-21.2	-20.1	-20.6 (0.3)	13	-0.8	4.2	1.9 (1.4)				
						I	Dentine	:					E	namel		
	n	min	max	Mean (1sd)	n	min	max	Mean (1sd)					n	min	max	Mean (1sd)
	12	7.9	11.8	9.2 (1.1)	12	-21.1	-20.0	-20.6 (0.4)					12	-15.4	-12.9	-14.3 (0.7)
Vufflens-la-Ville, En							Bone									
Sency	n	min	max	Mean (1sd)	n	min	max	Mean (1sd)	n	min	max	Mean (1sd)				
	12	8.4	10.0	9.1 (0.5)	12	-20.9	-20.3	-20.5 (0.2)	12	2.0	4.4	3.1 (0.7)				
				-		I	Dentine	:					E	namel		
	n	min	max	Mean (1sd)	n	min	max	Mean (1sd)					n	min	max	Mean (1sd)
	12	8.5	10.4	9.4 (0.7)	12	-21.5	-20.4	-20.8 (0.3)					12	-13.7	-12.8	-13.5 (0.3)
Tolochenaz, Le Boiron, La							Bone									
Caroline	n	min	max	Mean (1sd)	n	min	max	Mean (1sd)	n	min	max	Mean (1sd)				
	12	7.3	9.7	8.1 (0.7)	12	-20.4	-17.2	-18.2 (0.9)	11	1.2	8.9	4.3 (2.3)				
						Ι	Dentine	1					E	namel		
	n	min	max	Mean (1sd)	n	min	max	Mean (1sd)					n	min	max	Mean (1sd)
	10	7.3	10.1	8.7 (1.1)	10	-20.3	-16.4	-17.5 (0.7)					10	-13.3	-9.1	-10.3 (1.2)
p kruskal Wallis (bone)	n 37		<i>p</i> < 0	.001	n 37		<i>p</i> < 0.	001	n 36		<i>p</i> = 0	.002			· · · · ·	
P kruskal Wallis (dentine)	n 34		<i>p</i> = 0	.124	n 34		<i>p</i> < 0.	001					<i>p</i> kruskal Wallis (enamel)	N 34	p <	< 0.001

Table 7. Statistical parameters of isotope data for bone and dentine human collagen and apatite.

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plants, proportional to the duration and intensity of fertilization. According to the $\delta^{15}N$ ranges established by [70], even though a limited number of samples are present for the Early and Middle Bronze Age, the present data show a certain degree of homogeneity in $\delta^{15}N$ values for these two periods because most of them are between 3 and 4‰. Conversely, the results of the final phases of the Bronze Age show 1) a greater variability and 2) an enrichment in ¹⁵N compared to the previous chronological phases (Fig 3A). This could be due to an increase in the use of manure during the Final Bronze Age. Moreover, all the samples come from the same site which means the change in the isotopic signal can be followed over time, a change that could mainly reflect modifications in human agricultural practices because the settlement had

Table 8. Exact Wilcoxon-Pratt signed-rank test for bone and dentine collagen.

Collombey-Muraz, La Barmaz	Vufflens-la-Ville, En Sency	Tolochenaz, Le Boiron, La Caroline
δ^{13} C, $p = 0.835$	δ^{13} C, <i>p</i> = 0.019	$\delta^{13}C, p = 0.046$
δ^{15} N, <i>p</i> = 0.024	δ^{15} N, <i>p</i> = 0.066	δ^{15} N, <i>p</i> = 0.093

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Fig 3. Stable carbon and nitrogen isotope ratios for the archaeobotanical remains. Corrected values have been used for a) δ^{15} N results (-0.31‰ for the grains and +2.4‰ for the chaff to eliminate the charring effect) and b) δ^{13} C results (-0.11‰ for the grains and +1.9‰ for the chaff). A) Individual data and means (1SD) values are reported for C₃ plants (wheat and barley) and C₄ plants (foxtail and broomcorn millet) according to the chronological phases; B) Water status measured according to the ranges proposed by [104].

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a continuous chronology. Millets and legumes warrant particular attention. Not only are millets well represented in this and other coeval sites [44, 46, 118–121], but all the foxtail and broomcorn millet seeds of this settlement show δ^{15} N values higher than 5‰. These figures could suggest that these plants were well manured, supporting the importance of these crops in the local economy and, presumably, in the human and/or animal diet. Legumes, whose values are usually lower than those of cereal plants, are less sensitive to the effects of manuring. According to [70], only extremely intensive manuring (> 35 ton/ha for a prolonged duration) increases legume δ^{15} N considerably above 0‰. Here, pulses, mainly represented by beans, show values of up to 6.4‰, indicating a high level of fertilization. Such results seem to point to intensified agricultural activity and the increased impact of human actions on farming produce, supporting the idea of more intense agricultural practices during the Bronze Age. This result is in line with the increased demographic pressure suggested by the growing number of settlements and necropoles around Lake Geneva during that time.

An additional parameter to define agricultural and pastoral strategies is the δ^{15} N values from wild and domesticated herbivores. From these animal data, it is possible to estimate the values of the plants used to feed them. By inferring the herbivore forage isotope values from bone collagen it is possible to assess the stable isotope values of crops against those of forage. The estimated herbivore forage values represent a "sampling" of local vegetation to which crop values can be compared with to infer agricultural strategies and human practices like manuring and irrigation [69, 75]. The δ^{15} N values of the estimated forage suggest that the plants consumed by these animals do not fit within the intervals of the fertilized crops (Fig 4). Two hypotheses can therefore be proposed: the first is that the animals mainly consumed wild plants, likely from more humid or forested areas, while the second presupposes a separate cultivation of plants for fodder. If the latter proposition is retained, it is possible that these crops



Fig 4. Scatter plot of δ^{13} C and δ^{15} N bone collagen values for human, animal and botanical remains according to their chronological phases. C₃ plants include wheat and barley, C₄ plants include broomcorn and foxtail millets (representation of mean and 1SD for the plants). Abbreviations: TO = Chens sur Léman, Tougues.

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either underwent less intense fertilization compared to crops for human consumption or that they came from unmanured pastures.

Carbon values of botanical remains reflect the water conditions from which plants have grown [71, 104]. Taking into account the CO₂ changes that occurred throughout the Holocene, the plant water availability was estimated using the Δ^{13} C values (online resource: http:// web.udl.es/usuaris/x3845331/AIRCO2_LOESS.xls; [71]), making it possible to compare archaeological data over time. Although the quality and quantity is different according to each Bronze Age phase, the results highlight that barley, wheat and legumes did not suffer hydric stress (Fig 3B). Paths lined by small ditches and small artificial channel structures that canalized water towards fields were discovered in the site from which the carpological remains come from [47], suggesting that water was provided by humans through irrigation infrastructures. Usually, Δ^{13} C values for barley grains are $\approx 1\%$ higher than those for wheat grown in similar water conditions, mainly because of a difference in crop cycles [104]. In this study, barley shows lower Δ^{13} C values compared to wheat and pulses. It is, therefore, likely that barley was less watered than the other species. If the main crops were more tended to through irrigation, these results suggest that wheat and pulses were more important for cultivation than barley. In contrast to the archaeobotanical analysis of the site which indicates that barley was the main crop [122], this suggestion is, nevertheless, consistent with other local and western Switzerland results where wheat prevails [42, 119–121].

Despite the small archaeobotanical sample size making it difficult to present a global scenario for potential changes in farming practices over time, these results are comparable to those of other diachronic Bronze Age studies in southern Europe, where a similar surge in manuring practices and general good water conditions have also been observed [123].

Reconstructing dietary behaviors

Domestic and wild animal feedings. The δ^{15} N and δ^{13} C results for domestic herbivores (sheep/goat, cattle and pig) show no significant differences for carbon and nitrogen values. This reveals the global homogeneity of isotopic values throughout the Bronze Age (Fig 4). Notwithstanding, statistical differences occur for sulfur values (Table 5): the Rances animals have higher δ^{34} S values than those of La Barmaz and Chens-sur-Léman (δ^{34} S mean = 8.6‰ vs 4.2‰ and 3.3‰, respectively). Rances is situated further north than the other sites. It is possible that the environment where these animals lived was slightly different from the others, affecting the local values at the base of the food chain and not necessarily implying a diverse diet. All the animals, in fact, indicate a consumption of resources from a temperate environment, mostly constituted of C₃ type plants. As previously mentioned, the fodder for the domestic herbivores does not appear to have been manured, indicating that these animals could have consumed wild plants, or non-fertilized cultivated plants (Fig 4). Regardless, given that the animal results are all homogeneous, no differential livestock strategies are detected for the Bronze Age.

The three pike values, analyzed in order to understand the possible contribution of aquatic resources to the human diet, are in line with archaeological freshwater fish values in an Alpine environment [124].

The human diet. Throughout the sites and phases the local resources are similar, the three human groups can be directly compared. Any differences detected in the human diets must, therefore, depend on different dietary choices. Significant differences for δ^{13} C, δ^{15} N and δ^{34} S among the three groups are present, suggesting changes in dietary habits throughout the Bronze Age (Fig 4). These results are likely due to 1) a greater variability in food consumption over time and 2) the introduction of new resources in the diet at the end of the Bronze Age. The wide distribution of δ^{13} C values indicates diverse consumption of C_3 and C_4 plants, whereas the δ^{15} N and δ^{34} S ranges highlight different animal protein intake and, most likely, from different areas.

The bone collagen δ^{15} N and δ^{13} C values of the Early Bronze Age La Barmaz humans suggest a mixed terrestrial diet mainly based on C₃ plants and animal resources (Fig 4). This is supported by the offset between humans and animals, Δ^{13} C_{humans-animals} = 0.5‰ and Δ^{15} N_{humansanimals} = 3.7‰ (Table D in S1 Text) which are typical values falling within the ranges estimated between two consecutive trophic level. Furthermore, the quite narrow Δ^{13} C and Δ^{15} N spacings (Δ^{13} C = 1.1‰ and Δ^{15} N = 2.3‰) suggest that these individuals consumed similar foodstuffs. By applying a Bayesian model like FRUITS which takes into account the coeval isotopic data for animal and plant remains, the contribution of the different food sources to the diet can be better estimated. Fig 5A shows the scenario elaborated by FRUITS (S1 Table). Cereals like wheat and barley played a major role in the diet, representing 54.5%. They are followed by animal products, whose contribution is estimated at almost 30% (26.8%). The importance of animal foodstuff is also supported by the Δ^{13} C_{humans-plants} and Δ^{15} N_{humans-plants} offsets, which



Collombey, La Barmaz

Fig 5. The graphs a), c) and e) represent the contributions of foodstuffs as estimated by the Bayesian model FRUITS using $\delta^{13}C$ and $\delta^{15}N$ from human and animal bone collagen and C_3 and C_4 cereal plants (for the values entered refer to <u>S1 Table</u>). The graphs b), d) and f) illustrate the individual human bone collagen values and the mean isotope values of the main components of the available foodweb resources. C_3 and C_4 plant, freshwater fish and herbivore/pig values (with a herbivore diet) represent the means (±1SD) measured for each Bronze Age phase. Animal forage is evaluated by subtracting the isotopic offsets between plants and consumer collagen (-4.8‰ and -4‰ for the $\delta^{13}C$ and $\delta^{15}N$ animal means, respectively); 100% C_3 plants, 100% C_4 plants, 100% herbivore/pig meat and 100% pike are estimates of the mean isotopic composition of collagen values for consumers that are one trophic level above the respective resources (+4‰ for $\delta^{15}N$ and +0.8‰ for $\delta^{13}C$ for collagen and +4‰ for $\delta^{15}N$ and +4.8‰ for $\delta^{13}C$ for plants consumers). [†] MBA-FBA cereals. [‡] The animals which showed a herbivore diet are considered together for the three BA phases due to the limited number of individuals for the MBA (see the main text for further details). [§] FBA animals with a herbivore diet.

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equal 5.2‰ and 5‰, respectively, and are higher than expected from just C_3 plant consumption alone (Table D in S1 Text). This hypothesis is also confirmed by the scenario presented in Fig 5B, where the La Barmaz humans fit between the ranges of 100% C_3 plant diet and 100% herbivore and pig meat diet. In addition, legumes may have also contributed to the protein intake but as no data are available for these plants it is not possible to estimate their contribution. Little C_4 plants (14.9%) seem to have been consumed by this community.

Sulfur results support an intake of terrestrial resources. All the δ^{34} S values are below 6‰ and most of them are between 0‰ and 6‰, the typical range for the terrestrial environment. Furthermore, no significant correlation is observed between δ^{15} N and δ^{34} S values (Rho = -0.11, p = 0.70). Two individuals, BA5 and BA26, have negative δ^{34} S values. Considering that all the pikes have negative values, freshwater fish cannot be excluded from the diet of these two people. Moreover, δ^{15} N values are also quite enriched in ¹⁵N (BA5 = 8.4‰ and BA26 = 10.0‰). However, a significant consumption of freshwater fish is less likely for the rest of the group as the δ^{13} C and δ^{34} S values reflect a more terrestrial diet and, according to the FRUITS' model, the contribution of fish to the diet is very low (3.7%).

When considering the anthropological data, no dietary differences are recorded according to age and sex (Table C in <u>S1 Text</u>). Indeed, this suggests that sex and age probably did not lead to differential dietary practices. A similar assessment is proposed in northern Italy [22, 125] and in central France [126], suggesting an intra-group homogeneity in dietary patterns for the Early Bronze Age. However, some exceptions have been recorded, like in southern Germany [23] and in Poland, where small differences in favor of elder men suggest that mature adults had a diet more enriched in animal protein [52, 127].

The δ^{15} N and δ^{13} C bone collagen values from the Middle Bronze Age individuals of Vufflens indicate a mixed terrestrial diet, which would have been similar to those from La Barmaz. In order to estimate the $\Delta^{13}C$ and $\Delta^{15}N$ offsets between humans and animals of the Middle Bronze Age, the animals from the three phases which displayed a herbivore diet were grouped together. The Δ^{13} C_{humans-animals} (= 0.7‰) and Δ^{15} N_{humans-animals} (= 3.7‰) values are typical collagen offsets recorded between two adjacent trophic levels. The δ^{15} N and δ^{13} C human values display a limited range (Δ^{13} C = 0.6‰ and Δ^{15} N = 1.6‰). The dietary intake was, therefore, rather uniform within this community. According to FRUITS' model, it seems C₃ cereal plants were the main component of the diet (Fig 5C), with an estimated contribution equal to 57.6%. The animal protein intake is estimated at 25.6%. The $\Delta^{15}N_{humans-plants}$ offset is quite important, 5.9%, suggesting additional resources other than plant based. On the contrary, the $\Delta^{13}C_{humans-plants}$ offset is 4.3‰ which is slightly lower than the offset estimated between plants and their consumers (4.8‰). This scenario contrasts, to some extent, with the one observed at La Barmaz, where according to FRUITS, the C_3 plant contribution was lower than in Vufflens (54.5% vs. 57.6%) and animal intake higher (26.8‰ vs. 25.6‰). The differences are quite small and given that these estimates present some uncertainties (S1 Table), it is likely that minor differences in food

intake are detected with difficulty. Furthermore, there are some resources like pulses whose contribution is difficult to evaluate. Indeed, pulses values overlap with the C₃ plants, making their consumption even more difficult to estimate because their relative intake cannot be distinguished. However, the Vufflens individuals are above the range of 100% C_3 plant diet and within the range of 100% herbivore and pig meat diet (Fig 5D), suggesting a significant role of animal products in the diet of this community. In this case, an eventual consumption of legumes could have been masked by a considerable intake of animal protein. Additionally, given that no significant differences occur between the different Bronze Age animal groups and very few are present between the C_3 cereal plants of the two phases, the individuals of La Barmaz and Vufflens are directly comparable. Though both the carbon and nitrogen ranges mainly overlap, significant differences are observable for the δ^{15} N values (Table B in S1 Text, Fig 4), confirming a difference in terms of animal protein intake between the two communities. As for the C_4 plants, they were not a main component of the diet in the Vufflens humans, nor was freshwater fish (Fig 5C and 5D). Sulfur data confirm the main C_3 terrestrial origin of the resources consumed. The δ^{34} S mean is 3.1‰ with a limited distribution (Δ^{34} S = 2.4‰) and no significant correlation is observed between δ^{15} N and δ^{34} S (Rho = -0.08, *p* = 0.78). Like in the La Barmaz cemetery, no differences in food habits are recorded that correlate with the anthropological data and the funerary treatment. Moreover, the statistical analyses do not reveal any differences either (Table C in S1 Text).

The Final Bronze Age results from the Tolochenaz humans clearly differ from the two previous communities, particularly the carbon data. First, the δ^{13} C, δ^{15} N and δ^{34} S values of Tolochenaz are more widespread compared to those of La Barmaz and Vufflens, suggesting a more varied diet within this group. Then, the $\Delta^{13}C_{\text{humans-animals}}$ offset of 3% (min = -20.4% max = -17.2‰), highlights a mixed C_3 - C_4 diet, with a remarkable enrichment in ¹³C for the human values. This reflects C₄ plants like millets intake. As for the nitrogen, a Δ^{15} N_{humans-animals} offset of 2.5‰ indicates a rather limited consumption of animal proteins (Fig 4). This is confirmed by the FRUITS' model, where even if the main staple was C_3 cereal plants (56.1%), C_4 plants played an important role in the diet (21.6%), in addition to a less important animal product intake (18.8%). The freshwater fish contribution was minimal (3.3%) (Fig 5E). This scenario is confirmed in Fig 5F, where the Tolochenaz humans plot below the 100% herbivore and pig meat range, and are slightly to left of the 100% C₃ plant range because of the contribution of millets. Sulfur results support the main terrestrial origin of the resources consumed, confirmed by the absence of a significant correlation between δ^{15} N and δ^{34} S (Rho = 0.36, *p* = 0.33). No differences are evident when analyzing the results according to the individuals' associated grave goods. Nevertheless, only LeB3, differs from the general pattern. His/her δ^{13} C value of -20.4‰ is typical of a diet based on C₃ resources and the δ^{15} N is the highest, 9.7‰, suggesting a greater animal protein intake than for the rest of the group. Indeed, this result fits perfectly in the range of the 100% animal diet (Fig 5F). However, his/her δ^{34} S value (2.9‰) is consistent with the other Tolochenaz results. It is therefore not possible to ascertain whether this individual came originally from a different place or not. Moreover, since his/her sex and age were not evaluated because of the bad preservation of the remains and no special grave goods were present, it is difficult to formulate a hypothesis to explain the difference in diet. It is possible he/she came from another community, where different food habits could have been in place. Discussion about mobility is further approached in another paper, where additional strontium and oxygen isotope data are considered.

Reconstruction of dietary changes during the Bronze Age. This research shows differences in dietary practices both within and among communities throughout the Bronze Age in a region that is geographically restricted. Whilst during the first phases of the Bronze Age, barley, einkorn and wheat are the basis of the diet, during the Final Bronze Age broomcorn and foxtail millet are introduced, becoming an important component of the diet, as confirmed in the following phases at least for some individuals [60, 128]. This could be due to the introduction of new agricultural practices following new social and economic contacts with southern Europe societies where these crops were already present [20, 21, 25]. Another factor could be the intensification of anthropic activities on the environment due to population growth, among which would be deforestation and diversified land use-forestry, agriculture and pastoralism. These human activities could have strongly impacted soil conditions causing dryness, responsible for the decrease in soil fertility, which in turn could have favored the farming of more resistant crops, better adapted to harsh and extreme conditions, like millets. In addition, the influence of climate fluctuation during the Bronze Age needs to be considered. It could have contributed to the increase of soil aridity and pushed humans to look for and adopt new staple crops [129]. The combination of these events was responsible, on the one hand, for the development of alternative strategies for more efficient and fast agricultural production, and on the other hand, for the diffusion of new species. Even though in some areas, like in northern and central Italy, this trend appears earlier, from the Early-Middle Bronze Age transition [20, 21, 24, 25], these results confirm the general European and western Asian trend. Isotopic studies on humans and animals in France [126, 130], Germany [23, 131, 132], Austria [133], Hungary [134] Slovenia [135], Croatia [136] indicate millet consumption from the Final Bronze Age onwards. Switzerland, with a similar pattern, could be part of this "Euro-Asian wave" of new feeding practices, showcasing how this region is part of and well connected to the rest of Europe. The geomorphology of the area in which the sites are located, a wide Plateau surrounded by the Alps, is not a barrier to the diffusion of new activities and subsistence strategies. On the contrary, the mountains and the Plateau could in fact represent a source of unity because they are rich in raw materials, indispensable to the populations of the foothills, encouraging mobility and exchanges at different levels.

The consumption of animal products also changes over time. During the Early and Middle Bronze Age, meat and derived products seem to be quite significant, whereas during the Final Bronze Age they are less important. Low animal protein consumption at the end of the Bronze Age-beginning of the Iron Age is recorded in other European areas [137]. This decrease could be the result of a defined economic strategy directed to favor crop cultivation as opposed to animal husbandry, as suggested by the intense use of manure as well as the increase in crop diversity during the Final Bronze Age. The reason for these choices could be that population growth is easier to sustain through cultivated foodstuffs instead of animal meat production or the production of secondary animal products.

The three groups do not show any aquatic resource exploitation, despite being close to rivers and lakes. However, the occasional consumption of fish cannot be excluded. Its intake could have been so low that it would not be detected through isotope analysis.

Regarding potential differences in dietary patterns linked to individual social status, ethnological research has demonstrated that high social status privileges include differential access to food, with certain foodstuffs being exclusively set aside for rulers or the ruling lineage [138]. When comparing individuals in Early and Middle Bronze Age cemeteries with different grave goods, almost no differences in terms of diet and geographic origin appear [20, 22, 126], with few exceptions [139]. On the contrary, differences are observable from the end of the Bronze Age [21, 140, 141] and sometimes they seem more linked to the funerary structure than to the grave goods [136]. During the Iron Age, men buried with weapons often have diets with a higher animal protein component than other individuals [59, 132, 133, 142]. Among the communities studied here, high social status individuals have not been identified through the archaeological material except for those from Vufflens, where all the individuals likely belong to the local elite [31]. However, Vufflens is the only Middle Bronze Age community



Fig 6. Scatter plot of the δ^{13} C and δ^{15} N values of dentine (triangle) and bone (circle) collagen at the three sites. Data points are labelled with grave numbers. https://doi.org/10.1371/journal.pone.0245726.g006

isotopically studied to date in this area. Then, since the Middle Bronze Age data are similar to that of the Early Bronze Age, dietary trends seem consistent throughout the earlier Bronze Age periods and no specific foodstuffs appear to have been consumed by high-status individuals.

Juvenile vs. adult dietary patterns. The bone and dentine $\delta^{15}N$ and $\delta^{13}C$ collagen results indicate that diet did not differ significantly between childhood and adulthood (Fig 6). This means that the general food habits were not age dependent, but rather, were characteristic of each community. In fact, in La Barmaz and Vufflens the data suggest a prevalent C₃ diet from infancy, whereas in Tolochenaz the contribution of millets is highlighted from infancy, because both dentine and bone show high $\delta^{13}C$ values (Fig 6). Furthermore, as already observed, the La Barmaz and Vufflens bone collagen $\delta^{15}N$ and $\delta^{13}C$ results have a narrow distribution, suggesting consistent dietary choices within each group, contrary to Tolochenaz, whose $\delta^{13}C$ and $\delta^{15}N$ values show a wider distribution. These trends can also be observed for collagen dentine results. The Tolochenaz $\delta^{13}C$ and $\delta^{15}N$ ranges are larger than the La Barmaz and Vufflens ranges (excluding from the $\delta^{15}N$ values of La Barmaz the outlier BA23; S5 Fig). Consequently, for the Final Bronze Age, as opposed to the previous periods, both children and adults have access to a wide variety of food resources.

Two general patterns are evident in all sites. First, the dentine values range more widely than the bone ones. Second, the δ^{15} N values are higher in dentine than in bone, even if only La Barmaz shows statistical differences (p = 0.024) (Fig 6, Table 7). These two trend are in all likelihood linked to a greater consumption of animal protein during childhood. The $\Delta^{15}N_{dentine}$ bone individual values of La Barmaz range from -0.2‰ to 1.7‰, which can be explained through a greater meat and/or dairy food intake in childhood compared to adulthood. However, as similar trends have already been highlighted in other communities, other factors, especially physiological ones, may have caused the enrichment in ¹⁵N in dentine [143, 144]. Changes in δ^{15} N values can be also related to extended periods of breastfeeding, episodes of physiological stress and catabolism generated by starvation, long-term disease (infection, injury) or harsh climatic conditions [145–150]. In catabolic states, the organism uses amino acids from its own body tissues to synthesize new proteins, leading to an increase of δ^{15} N values [147, 151–153]. Elevated δ^{15} N levels could also result from a catabolism related to malnutrition [152, 154]. Enamel hypoplasia has been identified in several individuals (e.g., BA6, BA6a/b), but given its complex etiology, it is possible that malnutrition or other physiological stress resulted in fluctuations in the δ^{15} N collagen values of these individuals.

In terms of the $\Delta^{13}C_{dentine-bone}$ values, statistical differences are present in Vufflens and Tolochenaz (p = 0.019 and p = 0.046, respectively). The difference between the dentine and bone means are 0.3‰ in Vufflens and 0.6‰ in Tolochenaz. A value like 0.3‰ could just reflect the normal fluctuation over a person's lifetime, whilst 0.6‰ is quite considerable, since it is almost a step between two consecutive trophic levels. Particularly, LaC3 and LaC7 show wide offsets of 1.8‰ and 1.9‰, respectively. Even if for both individuals, bone and dentine values confirm a substantial millet intake, teeth show higher $\delta^{13}C$ compared to bone. It seems that millet was more important in childhood than in adulthood. This result is apparent in most individuals. While elevated $\delta^{15}N$ can represent both nutritional and physiological stress, a metabolic relationship with $\delta^{13}C$ is limited [155]. Thus, variations in $\delta^{13}C$ values reflect differential contributions of C_3 and C_4 plants. Isotope values do not seem to highlight any differences according to biological criteria such as age and sex. These elements appear therefore to have no impact on dietary choices.

Two individuals from Tolochenaz, LeB3 and LaC1, stand out because their diets differ from the rest of the community (Fig 6). LeB3 has already been discussed for the diet mainly based on C₃ plants as an adult, but no teeth are available to confirm this trend in childhood as well. Concerning LaC1, both bone and dentine were analyzed. The bone values indicate bad preservation and were therefore not included in the previous discussion. However, if the results are correct, he would be the only individual with a drastic dietary change during his life. During childhood he would have mainly had a C₃ diet ($\delta^{13}C = -20.3\%$), while in adulthood he would have had a mixed C₃-C₄ diet ($\delta^{13}C = -17.6\%$). He was a mature adult man, with a callus bone in the right ulna resulting from a fracture [35]. Unfortunately, the discussion for this individual can go no further for obvious reasons, but some interesting questions arise. If the results are acceptable, why is he the only one with a dietary shift? Even if a definitive answer is not possible, this study identifies the presence of individuals with different dietary habits, likely linked to mobility events. Indeed, this result may support exchanges between different communities with diverse eating traditions. Investigations on strontium and oxygen isotopes provide further details about this individual's life [156].

The apatite data. Carbon in apatite is a useful proxy to detect the contribution of low trophic level food, representing a supplementary trophic marker [157–159]. It helps determine marine vs. terrestrial diets and C₃ vs. C₄ plant intake [80, 86, 157, 160]. Combining collagen and apatite data through the apatite carbon isotopic spacing, $\Delta^{13}C_{ap-coll}$, it is possible to assess whether carbon enriched food sources are protein-depleted or protein-rich. In the case of a



Fig 7. Dentine nitrogen isotope ratios and enamel-dentine collagen spacing for humans from the three sites in western Switzerland.

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monoisotopic diet–that is, when δ^{13} C from all macronutrients originates from the same main food source–a spacing of ca. 8.4‰ (13.4–5‰) can be assumed between carbon in dentine and in enamel ($\Delta^{13}C_{ap-coll}$) because (a) the $\delta^{13}C_{enamel}$ value in tooth apatite is enriched of ca. 13.4 ± 1.0‰ when compared to the actual diet [82, 84, 85] and (b) a difference of ca. 5‰ between the δ^{13} C of the diet and the collagen can be estimated if the δ^{13} C value of dietary protein and energy are the same [79]. The $\Delta^{13}C_{ap-coll}$ is expected to be higher than 8.4‰ if the diet is mainly based on C₄ carbohydrates and C₃ proteins. On the contrary, a lower spacing is expected if there is an important consumption of C₃ carbohydrates and marine protein.

In this study, the $\Delta^{13}C_{ap-coll}$ values range from 5.3‰ to 8.0‰. The La Barmaz mean is 6.2‰ \pm 0.5‰ (min = 5.3‰, max = 7.1‰). The Vufflens mean is 7.3‰ \pm 0.4‰ (min = 6.6‰ max = 7.8‰). Finally, the Tolochenaz mean is 7.4‰ \pm 0.7‰ (min = 6.9‰ max = 8.0‰). According to these results, C₃ plants and animal proteins were consumed in greater proportion during childhood at the beginning of the Bronze Age, whereas the intake of C₄ plants increased during the later phases of the Bronze Age at the expense of animal protein. This result is confirmed when combining the $\Delta^{13}C_{ap-coll}$ with the $\delta^{15}N$ in dentine collagen. Indeed, the results suggest a diet mainly composed of C₃ protein foods and C₃ energy food sources in the Early and Middle Bronze Age. During the Final Bronze Age, the main protein intake came from a C₃ environment but the bulk of the diet included low-protein C₄ foodstuffs (Fig 7). Furthermore, no correlations occur between the $\Delta^{13}C_{ap-coll}$ spacing and the dentine $\delta^{15}N$ values in La Barmaz and Vufflens. However, in Tolochenaz a negative significant correlation is evident suggesting the presence of a strong trophic level effect that varies with the individuals (Fig 7).

In order to evaluate the changes in C₄ contributions during the Bronze Age, the model elaborated by [161] and [86, 157] was applied (%C₄ = $(-25 - (\delta^{13}C_{\text{bone apatite}} - 9.4)) / 15 \times 100$). However, this model was developed from experimental studies on $\delta^{13}C_{\text{bone apatite}}$ and $\delta^{13}C_{\text{bone collagen}}$. Tooth enamel apatite ratios are not equivalent to bone apatite ratios [82, 162], therefore, the $\delta^{13}C_{\text{enamel}}$ values were adjusted. Some studies have estimated the offsets between $\delta^{13}C_{\text{bone apatite}}$ and $\delta^{13}C_{\text{bone apatite}}$ and $\delta^{13}C_{\text{bone apatite}}$.

these results, an average value of + 1.8‰ has been added to $\delta^{13}C_{enamel}$ values [165] before calculating the proportion of C₄ resources consumed. Although the results should be considered with caution due to analytical uncertainty and to inter-individual variability in the estimated enamel to bone spacing, they suggest a broad difference in the proportion of C₄ plants consumed during childhood by individuals buried at La Barmaz and Vufflens (respectively an estimate of ca. 20 and 26%) and by individuals buried at Tolochenaz (almost half of the diet derived from C₄ plants, ca. 47%). Thus, $\delta^{13}C_{enamel}$ value confirms first that the dietary patterns detected in adulthood throughout the Bronze Age are similar to those in childhood and, second, that C₄ plants were directly consumed by humans.

Conclusions

This paper presents for the first time a complex multi-isotopic and bioarchaeological study on Bronze Age western Switzerland. This area is a natural and economic crossroads between central Europe and the Mediterranean area and this study contributes to the reconstruction of human dietary changes during the Bronze Age and sheds light on the complexity of human behavior, filling a gap for central Europe.

The botanical data suggest an increased impact of anthropic activities during the Bronze Age. The manuring practices seemed to play an important role in the farming systems, particularly at the end of the Bronze Age. There was greater diversity in cereal cultivation, highlighted by the adoption of new staple crops such as millet varieties. Moreover, plant isotopic data indicate that they grew in good hydric conditions, likely directly managed by humans. As for domestic herbivores, no different herding strategies have been detected throughout the Bronze Age. The fodder they consumed was based on wild plants and/or non-fertilized cultivated plants. Based on a reliable isotopic baseline of coeval local food sources, the human data show that dietary habits changed during the Bronze Age. The staple crops were wheat, barley and pulses during the first phases of the Bronze Age, whilst millets, better adapted to poor soil conditions, were adopted at the end of the Bronze Age. It could be the result of several factors. Among others, exchanges with communities in southern Europe where these crops were already present, like northern Italy, the increasing in dryness caused by the overexploitation of the soils, without excluding the impact of climatic fluctuation along the Bronze Age. Furthermore, there is less animal protein intake during the Final Bronze Age than in previous periods. It is likely that the demographic increase recorded during this phase led to a change in economic strategies, favoring crop cultivation instead of animal husbandry. Further investigations on Final Bronze Age communities could provide more details on this trend.

The comparison between teeth and bone data suggests that, even though a general greater consumption of animal products in childhood than in adulthood is generally highlighted, the main patterns identified in adults are confirmed in juveniles. Thus, the general food habits were not age dependent, but rather, were characteristic of each community. However, some exceptions are present and these individuals could have had a different origin and therefore different dietary habits linked to their local origins.

This research highlights the importance studying the entire trophic chain, including archaeobotanical samples that are still rare in European isotopic research, because this provides crucial details for the reconstruction of the paleoenvironment, agricultural strategies and human dietary choices. Furthermore, multi-sampling analysis of human remains enabling cross-sectional evaluation facilitates tracking dietary changes throughout an individual's lifespan. This study is the first step to understand how protohistoric populations adapted and modified the landscape where they lived in a limited but well-defined area at the heart of Europe.

Supporting information

S1 Text. Table A. Botanical and animal remains analyzed in this study. **Table B.** Bone and dentine collagen and enamel apatite Wilcoxon Mann-Whitney exact text; **Table C.** Bone and dentin collagen Wilcoxon Mann-Whitney exact text according to biological parameters (age and sex); **Table D.** Offsets calculated between plants, human and animal bone collagen. (DOCX)

S1 Table. Bayesian modelling of dietary patterns using FRUITS software. Data entry and results.

(XLSX)

S1 Fig. Collombey-Muraz, La Barmaz map of the cemetery with the individuals analyzed (designed by M. Honegger).

(TIF)

S2 Fig. Vufflens-la-Ville, En Sency: Map of the cemetery with the individuals analyzed (Reprinted but modified from [31] under a CC BY license, with permission from [CAR], original copyright [2005]).

(TIF)

S3 Fig. Tolochenaz, La Caroline: Map of the cemetery with the individuals analyzed, LaC (Reprinted but modified from [35] under a CC BY license, with permission from [CAR], original copyright [2019]).

(TIF)

S4 Fig. Box-plot showing the $\delta^{13}C_{enamel}$ values for Collombey-Muraz, La Barmaz, Vufflens-la-Ville, En Sency and Tolochenaz, Le Boiron, La Caroline humans. (TIF)

S5 Fig. Box-plots showing the stable isotope bone and teeth results for the $\delta^{13}C_{coll}$, $\delta^{15}N_{coll}$ for Collombey-Muraz, La Barmaz, Vufflens-la-Ville, En Sency and Tolochenaz, Le Boiron, La Caroline humans.

(TIF)

S1 File. (PDF)

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References

- 1. Kristiansen K. Europe before History. Cambridge: Cambridge University Press; 1998.
- 2. Stika H-P, Heiss AG. Plant cultivation in the Bronze Age. In: Fokkens H, Harding A, editors. The Oxford handbook of the European Bronze Age. Oxford: Oxford University Press; 2013. pp. 348–369.
- 3. Bartosiewicz L. Animals in Bronze age Europe. In: Fokkens H, Harding AF, editors. The Oxford Handbook of the european Bronze Age. Oxford: Oxford University Press; 2013. pp. 328–347.
- Rösch M, Fischer E, Kleinmann A, Lechterbeck J, Tserendorj G, Wick L. Bronzezeitliche Landnutzung im diachronen Vergleich—Fallbeispiele aus Südwestdeutschland. Arbetsberichte zur Bodendenkmalpfl Brand. 2014; 26: 13–27.
- Nikulka F. Archäologische Demographie. Leiden: Sidestone Editions; 2016. <u>https://doi.org/10.1007/s00402-016-2409-9 PMID: 26837222</u>
- Magny M, Maise C, Jacomet S, Burga AC. Environnement et économie de subsistance. In: Hochuli S, Niffele U, Rychner V, editors. La Suisse du Paléolithique à l'aube du Moyen-Age De l'Homme de Néandertal à Charlemagne, 3 Age du Bronze. Basel: Archäologie Schweiz; 1998. pp. 135–170.
- Mercuri AM, Accorsi CA, Bandini Mazzanti M, Bosi G, Cardarelli A, Labate D, et al. Economy and environment of Bronze Age settlements—Terramaras—On the Po Plain (Northern Italy): First results from the archaeobotanical research at the Terramara di Montale. Veg Hist Archaeobot. 2006; 16: 43–60.
- Cremaschi M, Pizzi C, Valsecchi V. Water management and land use in the terramare and a possible climatic co-factor in their abandonment: The case study of the terramara of Poviglio Santa Rosa (northern Italy). Quat Int. 2006; 151: 87–98.
- 9. Kristiansen K. Bronze age identities. From social to cultural and ethnic identity. In: McInerney J, editor. A companions to ethynicity in the Ancient Mediterranean. John Wiley & Sons; 2014. pp. 81–96.
- Radivojević M, Roberts BW, Pernicka E, Stos-Gale Z, Martinón-Torres M, Rehren T, et al. The Provenance, Use, and Circulation of Metals in the European Bronze Age: The State of Debate. J Archaeol Res. 2019; 27: 131–185.
- Cattin F, Guénette-Beck B, Curdy P, Meisser N, Ansermet S, Hofmann B, et al. Provenance of Early Bronze Age metal artefacts in Western Switzerland using elemental and lead isotopic compositions and their possible relation with copper minerals of the nearby Valais. J Archaeol Sci. 2011; 38: 1221– 1233.
- David-Elbiali M. Le passage des Alpeso: voyages et échanges entre l'Italie et la Suisse (2200–700 av. J.-C.). In: Lehoerff A, Talon M, editors. Movement, Exchange and Identity in Europe in the 2nd and 1st millenia BC. Oxford: Oxbow Books; 2017. pp. 290–304.
- David W, David-Elbiali M, de Marinis RC, Rapi M. Le Bronze moyen et récent en Italie du Nord, Allemagne du Sud et Suisse et corrélation des systèmes chrono-culturels. In: Lachenal T, Mordant C, Nicolas T, Véber C, editors. Le Bronze moyen et l'origine du Bronze final en Europe occidentale (XVIIe–XIIIe siècle av J-C). Strasbourg: AVAGE; 2017. pp. 565–600.
- Allentoft ME, Sikora M, Sjögren KG, Al. E. Population genomics of Bronze Age Eurasia. Nature. 2015; 522: 167–172. https://doi.org/10.1038/nature14507 PMID: 26062507
- 15. Knipper C, Mittnik A, Massy K, Kociumaka C, Kucukkalipci I, Maus M, et al. Female exogamy and gene pool diversification at the transition from the Final Neolithic to the Early Bronze Age in central

Europe. Proc Natl Acad Sci UA. 2017; 114: 10083–10088. https://doi.org/10.1073/pnas.1706355114 PMID: 28874531

- Olalde I, Brace S, Allentoft ME, et al. The Beaker phenomenon and the genomic transformation of northwest Europe. Nature. 2018; 555: 190–196. <u>https://doi.org/10.1038/nature25738</u> PMID: 29466337
- David-Elbiali M, Venturino Gambari M. Artisans métallurgistes de l'age du Bronze à travers les Alpes? Bull d'études préhistoriques archéologiques alpines. 2016; 27: 171–185.
- 18. David-Elbiali M. La Suisse occidentale au IIe millénaire av. J.-C.: chronologie, culture, intégration européenne. Cah d'archéologie Rom. 2000;80.
- David-Elbiali M, Kramar C, Studer J. L'Age du Bronze. In: Gallay A, editor. Des Alpes au Léman Images de la préhistoire. Gollion: Infolio; 2006. pp. 191–261.
- Tafuri MA, Craig OE, Canci A. Stable isotope evidence for the consumption of millet and other plants in Bronze Age Italy. Am J Phys Anthropol. 2009; 139: 146–53. <u>https://doi.org/10.1002/ajpa.20955</u> PMID: <u>19051259</u>
- Tafuri MA, Rottoli M, Cupito M, Pulcini ML, Tasca G, Carrara N, et al. Estimating C4 plant consumption in Bronze Age Northeastern Italy through stable carbon and nitrogen isotopes in bone collagen. Int J Osteoarchaeol. 2018; 28: 131–142.
- 22. Varalli, Moggi-Cecchi J, Dori I, Boccone S, Bortoluzzi S, Salzani P, et al. Dietary continuity vs. discontinuity in Bronze Age Italy. The isotopic evidence from Arano di Cellore (Illasi, Verona, Italy). J Archaeol Sci Reports. 2016; 7: 104–113.
- 23. Koch JK, Kupke K. Life course-reconstruction of mobile individuals in an Early Bronze Age society in Central Europe. Concept of the project and first results to the cemetery of Singen (Germany). In: Kaiser E, Burger J, Schier W, editors. Population dynamics in Pre- and Early History New Approaches by using Stable Isotopes and Genetics. Boston: De Gruyte Berlin; 2012.
- 24. Varalli, Moggi-Cecchi J, Moroni A, Goude G. Dietary Variability During Bronze Age in Central Italy: First Results. Int J Osteoarchaeol. 2016; 26: 431–446.
- 25. Varalli A. Ricostruzione delle strategie di sussistenza in Italia durante l'età del Bronzo: il contributo delle analisi isotopiche. University of Aix-Marseille, University of Florence. 2015.
- 26. David-Elbiali M. Retour vers la Walliser Kultur/culture du Valais et réexamen critique de la culture du Rhône. Bull d'études préhistoriques archéologiques alpines. 2019; 29–30: 137–144.
- 27. Della Casa P. Switzerland and the central Alps. In: Fokkens H, Harding A, editors. The Oxford Handbook of the European Bronze Age. Oxford: Oxford University Press; 2013. pp. 706–722.
- Favre S, Mottet M. Le site du Petit-Chasseur (Sion, Valais) 9. Dolmens M XII et M XIII, approche des différents niveaux préhistoriques. Cah d'archéologie Rom. 2011;123.
- **29.** Gubler R. Spiez-Einigen, Holleeweg 3. Gräber am Übergang zwischen Früh- und Mittelbronzezeit. Archäologie Bern. 2010.
- 30. Honegger M. Données récentes sur les nécropoles du Néolithique moyen I et du Bronze ancien de Barmaz I (Collombey-Muraz, Valais). Aspects culturels et religieux: témoignages et évolution de la préhistoire à l'an mil Actes du 7e colloque international sur les Alpes dans l'Antiquité (Châtillon, Vallée d'Aoste, 11–13 mars 1994). 1995. pp. 53–66.
- Mariéthoz F, David-Elbiali M, Eades S, Girod F, Guélat M, Northover P, et al. Enquête autour d'un tumulus de l'âge du Bronze: Vufflens-la-Ville, En Sency (VD, Suisse). Cah d'archéologie Rom. 2005;100.
- Boisaubert J-L, Bugnon D, Mauvilly M. Archéologie et autoroute A1, destins croisés. 25 années de fouilles en terres fribourgeoises, premier bilan (1975–2000). Fribourg: Academic Press Fribourg; 2008.
- 33. Wüthrich S. Colombier NE, Plantées de Rive. Annu d'Archéologie Suisse. 2014; 97: 207.
- Beeching A. Le Boiron: une nécropole du Bronze final près de Morges (Vaud-Suisse). Cah d'archéologie Rom. 1977; 11: 1–202.
- Gallay A, Burri-Wyser E, Menna F, David-Elbiali M. Tolochenaz (VD)—La Caroline. Du Mésolithique à l'epoque romaine en passant pour la nécropole du Boiron. Cah d'archéologie Rom. 2019; 168: 1–189.
- **36.** Schaer A. Die Bronzehand von Prêles, Kanton Bern (Schweiz). Tagungen des Landesmuseums für Vor Halle. 2019; 21: 301–320.
- 37. David-Elbiali M, Hafner A. Gräber, Horte und Pfahlbauten zwischen Jura und Alpen—Die Entwicklung elitärer sozialer Strukturen in der frühen Bronzezeit der Westschweiz. In: Meller H, Bertemes F, editors. Der Griff nach den Sternen: wie Europas Eliten zu Macht und Reichtum kamen. Halle: Landesamt für Denkmalpflege und Archäologie Sachsen-Anhalt, Landesmuseum für Vorgeschichte; 2010. pp. 217–238.

- Cattin F, Guénette-Beck B, Besse M, Serneels V. Lead isotopes and archaeometallurgy. Archaeol Anthropol Sci. 2009; 1: 137–148.
- Perucchetti L, Bray P, Dolfini A, Pollard AM. Physical Barriers, Cultural Connections: Prehistoric Metallurgy across the Alpine Region. Eur J Archaeol. 2015; 18: 599–632.
- 40. Cardarelli A. La necropoli della terramara di casinalbo. Firenze: All'insegna del Giglio; 2014.
- 41. David-Elbiali M, Studer J. Réflexion sur l'économie à l'âge du Bronze en Suisse: autarcie vivrière et échange de biens de prestige? In: Besse M, Stahl-Gretsch LI, Curdy P, editors. ConstellaSion: hommage à Alain Gallay. Lausanne: Cahiers d'archéologie romande et Société préhistorique française; 2003. pp. 267–272.
- 42. Kühn M, Heitz A. Vegetation history and plant economy in the Circum-Alpine region Bronze Age and early Iron Age environments: stability or major changes. In: Menotti F, editor. The end of the lakedwellings in the Circum-Alpine region. Oxford: Oxbow Books; 2015. pp. 125–178.
- **43.** Jacquat C, Studer J. L'âge du Bronze, naturellement! Le monde végétal et animal à Hauterive-Champreveyres NE. Archéologie suisse. 1999; 22: 22–26.
- Castella D, Buard J-F, David-Elbiali M, Jacquat C, Northover PJ, Olive C. L'habitat de l'âge du Bronze moyen de Payerne «En Planeise» (canton de Vaud, Suisse). Cah d'archéologie Rom. 2012; 133: 237–245.
- **45.** Jacomet S, Brombacher C. Geschichte der Flora in der Regio Basiliensis seit 7500 Jahren: Ergebnisse von Untersuchungen pflanzlicher Makroreste aus archäologischen Ausgrabungen. Mitteilungen der Naturforschenden Gesellshaften beider Basel. 2009; 11: 27–106.
- Jacquat C. Hauterive-Champréveyres 2. Les plantes de l'âge du Bronze: contribution à l'histoire de l'environnement et de l'alimentation. Archéologie neuchâteloise. 1989; 8: 1–113.
- Cousseran S, Néré E. L'agglomération protohistorique de Chens-sur-Léman. Un modèle d'habitat inédit. Archéopages. 2014; 40: 36–47.
- 48. Studer J. La faune des niveaux du Bronze moyen. Cah d'archéologie Rom. 2019; 175: 255–260.
- 49. Schibler J, Studer J. Elevage et chasse à l'âge du Bronze en Suisse. In: Hochuli S, Niffeler U, Rychner V, editors. La Suisse du Paléolithique à l'aube du Moyen-Age De l'Homme de Néandertal à Charle-magne, 3 Age du Bronze. Basel: Archäologie Schweiz; 1998. pp. 171–191.
- 50. Studer J, Portmann. La faune. Cah d'archéologie Rom. 2011; 122: 217–227.
- Hafner A, Schwörer C. Vertical mobility around the high-alpine Schnidejoch Pass. Indications of Neolithic and Bronze Age pastoralism in the Swiss Alps from paleoecological and archaeological sources. Quat Int. 2018; 484: 3–18.
- David-Elbiali M. Soziale Ungleichheit in bronzezeitlichen Frauen- und Männergräbern Europas. In: Hansen S, Müller J, editors. Rebellion and Inequality in Archaeology. Bonn: Rudolf Habelt Verlag; 2017. pp. 205–249.
- 53. Araus JL, Febrero A, Buxo R, Camalich MD, Martin D, Molina F, et al. Changes in carbon isotope discrimination in grain cereals from different regions of the western Mediterranean Basin during the past seven millennia. Palaeoenvironmental evidence of a differential change in aridity during the late Holocene. Glob Chang Biol. 1997; 3: 107–118.
- 54. Farquhar GD, Ehleringer JR, Hubick KT. Carbon Isotope Discrimination and Photosynthesis. Annu Rev Plant Physiol Plant Mol Biol. 1989; 40: 503–537.
- 55. Tieszen LL. Natural variations in the carbon isotope values of plants: implications for archaeology, ecology and palaeoecology. J Archaeol Sci. 1991; 18: 227–248.
- DeNiro MJ, Epstein S. Influence of diet on the distribution of nitrogen isotopes in animals. Geochim Cosmochim Acta. 1981; 45: 341–351.
- Schoeninger MJ, DeNiro MJ. Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. Geochim Cosmochim Acta. 1984; 48: 625–639.
- 58. Richards MP, Fuller BT, Hedges REM. Sulphur isotopic variation in ancient bone collagen from Europe: implications for human palaeodiet, residence mobility, and modern pollutant studies. Earth Planet Sci Lett. 2001; 191: 185–190.
- 59. Moghaddam N, Müller F, Hafner A, Lösch S. Social stratigraphy in Late Iron Age Switzerland: stable carbon, nitrogen and sulphur isotope analysis of human remains from Münsingen. Archaeol Anthropol Sci. 2016; 8: 149–160.
- Moghaddam N, Müller F, Lösch S. A bioarchaeological approach to the Iron Age in Switzerland: stable isotope analyses (δ13C, δ15N, δ34S) of human remains. Archaeol Anthropol Sci. 2018; 10: 1067– 1085.
- Nehlich O. The application of sulphur isotope analyses in archaeological research: A review. Earth-Science Rev. 2015; 142: 1–17.

- 62. Minagawa M, Wada E. Stepwise enrichment of 15N along food chains: Further evidence and the relation between δ15N and animal age. Geochim Cosmochim Acta. 1984; 48: 1135–1140.
- Bocherens H, Drucker D. Trophic level isotopic enrichment of carbon and nitrogen in bone collagen: Case studies from recent and ancient terrestrial ecosystems. Int J Osteoarchaeol. 2003; 13: 46–53.
- 64. Bogaard A, Styring A, Ater M, Hmimsa Y, Green L, Stroud E, et al. From traditional farming in Morocco to early urban agroecology in Northern Mesopotamia: combining present-day arable weed surveys and crop isotope analysis to reconstruct past agrosystems in (semi-)arid regions. Environ Archaeol. 2018; 23: 303–322.
- Heaton THE, Baruch S, Madeline S, Robertson C. Potential canopy influences on the isotopic composition of nitrogen and sulphur in atmospheric deposition. Oecologia. 1997; 109: 600–607. https://doi. org/10.1007/s004420050122 PMID: 28307345
- Farquhar GD, Hubick HT, Condon AG, Richards RA. Carbon isotope fractionation and water use efficiency. In: Rundel PW, Ehleringer JR, Nagy KA, editors. Stable isotopes in ecological research. New York: Springer-Verlag; 1989. pp. 22–40.
- Prado P, Vergara C, Caiola N, Ibáñez C. Influence of salinity regime on the food-web structure and feeding ecology of fish species from Mediterranean coastal lagoons. Estuar Coast Shelf Sci. 2014; 139: 1–10.
- Körner C, Farquhar GD, Wong SC. Carbon isotope discrimination by plants follows latitudinal and altitudinal trends. Oecologia. 1991; 88: 30–40. https://doi.org/10.1007/BF00328400 PMID: 28312728
- 69. Bogaard A, Fraser R, Heaton THE, Wallace M, Vaiglova P, Charles M, et al. Crop manuring and intensive land management by Europe's first farmers. Proc Natl Acad Sci USA. 2013; 110: 12589–94. https://doi.org/10.1073/pnas.1305918110 PMID: 23858458
- **70.** Fraser R, Bogaard A, Heaton T, Charles M, Jones G, Christensen BT, et al. Manuring and stable nitrogen isotope ratios in cereals and pulses: towards a new archaeobotanical approach to the inference of land use and dietary practices. J Archaeol Sci. 2011; 38: 2790–2804.
- Ferrio JP, Araus JL, Buxó R, Voltas J, Bort J. Water management practices and climate in ancient agriculture: Inferences from the stable isotope composition of archaeobotanical remains. Veg Hist Archaeobot. 2005; 14: 510–517.
- Ferrio JP, Voltas J, Alonso N, Araus JL. Reconstruction of climate and crop conditions in the past based on the carbon isotope signature of archaeobotanical remains. Terr Ecol. 2007; 1: 319–332.
- 73. Flohr P, Müldner G, Jenkins E. Carbon stable isotope analysis of cereal remains as a way to reconstruct water availability: Preliminary results. Water Hist. 2011; 3: 121–144.
- Goude G, Fontugne M. Carbon and nitrogen isotopic variability in bone collagen during the Neolithic period: Influence of environmental factors and diet. J Archaeol Sci. 2016; 70: 117–131.
- 75. Fiorentino G, Ferrio JP, Bogaard A, Araus JL, Riehl S. Stable isotopes in archaeobotanical research. Veg Hist Archaeobot. 2014; 24: 215–227.
- 76. Hillson SW. Dental Pathology. In: Katzenberg A, Saunders SR, editors. Biological Anthropology of Human Skeleton. New York: Wiley Liss; 2000. pp. 249–286. <u>https://doi.org/10.1517/14656566.1.5.</u> 1053 PMID: 11249495
- 77. Scheuer L, Black S. Developmental juvenile osteology. Oxford: Elsevier Academic Press; 2000.
- Hedges REM, Clement JG, Thomas CDL, O'connell TC. Collagen turnover in the adult femoral midshaft: modeled from anthropogenic radiocarbon tracer measurements. Am J Phys Anthropol. 2007; 133: 808–16. https://doi.org/10.1002/ajpa.20598 PMID: 17405135
- 79. Ambrose SH, Norr L. Experimental evidence for the relationship of the carbon isotope ratios of whole diet and dietary protein to those of bone collagen and carbonate. In: Lambert JB, Grupe G, editors. Prehistoric human bone Archaeology at the molecular level. Berlin: Springer-Verlag; 1993. pp. 1–37.
- Kellner CM, Schoeninger MJ. A Simple Carbon Isotope Model for Reconstructing Prehistoric Human Diet. Am J Phys Anthropol. 2007; 133: 1112–1127. <u>https://doi.org/10.1002/ajpa.20618</u> PMID: 17530667
- Schwarcz HP. Some biochemical aspects of carbon isotopic paleodiet studies. In: Ambrose SH, Katzenberg MA, editors. Biogeochimical approaches to paleodietary analysis. New York: Plenum Publishers; 2000. pp. 189–211.
- Warinner C, Tuross N. Alkaline cooking and stable isotope tissue-diet spacing in swine: archaeological implications. J Archaeol Sci. 2009; 36: 1690–1697.
- Howland MR, Corr LT, Young SM, AI E. Expression of the dietary isotope signal in the compound-specific δ13C values of pig bone lipids and amino acids. Int J Osteoarchaeol. 2003; 13: 54–65.

- Passey BH, Robinson TF, Ayliffe LK, Cerling TE, Sponheimer M, Dearing MD, et al. Carbon isotope fractionation between diet, breath CO2, and bioapatite in different mammals. J Archaeol Sci. 2005; 32: 1459–1470.
- 85. Salesse K. Archéo-biogéochimie isotopique, Reconstitutions des régimes alimentaires et des schémas de mobilité, et interactions bio-culturelles. Les sépultures plurielles de la Région X de la Catacombe des Saints Pierre et Marcellin (Rome, Ier-IIIe s ap. J.-C.). Ph.D. Thesis, The University of Bordeaux. 2015.
- 86. Ambrose SH, Butler BM, Hanson DB, Hunter-Anderson RL, Krueger HW. Stable isotopic analysis of human diet in the Marianas Archipelago, Western Pacific. Am J Phys Anthropol. 1997; 104: 343–361. https://doi.org/10.1002/(SICI)1096-8644(199711)104:3<343::AID-AJPA5>3.0.CO;2-W PMID: 9408540
- Harrison RG, Katzenberg MA. Paleodiet studies using stable carbon isotopes from bone apatite and collagen: examples from Southern Ontario and San Nicolas Island, California. J Anthropol Archaeol. 2003; 22: 227–244.
- **88.** Lee-Thorp JA, Sealy JC, Van der Merwe NJ. Stable Carbon Isotope Ratio Differences Between Bone Collagen and Bone Apatite and their Relationship to Diet. J Archaeol Sci. 1989; 16: 585–599.
- Acsádi G, Nemeskéri I. History of human life span and mortality. History of human life span and mortality. Budapest: Akadémiai Kiadó; 1970.
- Bruzek J. A method for visual determination of sex, using the human hip bone. Am J Phys Anthropol. 2002; 117: 157–168. https://doi.org/10.1002/ajpa.10012 PMID: 11815949
- Bruzek J. Fiabilité des procédés de détermination du sexe à partir de l'os coxal Implications à l'étude du dimorphisme sexuel de l'Homme fossile. Ph.D. Thesis, Muséum National d'Histoire Naturelle. 1991.
- 92. Miles AEW. Dentition in the Estimation of Age. J Dent Res. 1963; 42: 255–263.
- Schmitt A. Une nouvelle méthode pour estimer l'âge au décès des adultes à partir de la surface sacropelvienne iliaque. Bull Mem Soc Anthropol Paris. 2005; 17: 89–101.
- Moorrees CFA, Fanning EA, Hunt EE. Formation and resorption of three deciduous teeth in children. Am J Phys Anthropol. 1963; 21: 205–213. https://doi.org/10.1002/ajpa.1330210212 PMID: 14110696
- 95. Moorrees CF, Fanning E a, Hunt EE. Age Variation of Formation Stages for Ten Permanent Teeth. J Dent Res. 1963; 42: 1490–502. https://doi.org/10.1177/00220345630420062701 PMID: 14081973
- 96. Ubelaker DH. The estimation of age at death from immature human bone. In: Thomas CC, editor. Age Markers in the Human Skeleton. Illinois: Springfield; 1989. pp. 55–70.
- 97. David-Elbiali M, David W. Le Bronze ancien et le début du Bronze moyen: cadre chronologique et liens culturels entre l'Europe nord-alpine occidentale, le monde danubien et l'Italie du Nord. In: Richard A, Barral P, Daubigney A, Kaenel G, Mordant C, Piningre JF, editors. L'isthme européen Rhin-Saône-Rhône dans la Protohistoire: approches nouvelles en hommage à Jacques-Pierre Millotte. Besançon: Presses Université de Franche-Comté; 2009. pp. 311–340.
- 98. David-Elbiali M. La chronologie nord-alpine du Bronze final (1200–800 av. J.-C.): entre métal, céramique et dendrochronologie. In: Leclercq W, Warmenbol E, editors. Echanges de bons procédés: la céramique du Bronze final dans le nord-ouest de l'Europe. Bruxelles: CReA-Patrimoine; 2013. pp. 181–197.
- Longin R. New Method of Collagen Extraction for Radiocarbon Dating. Nature. 1971; 230: 241–242. https://doi.org/10.1038/230241a0 PMID: 4926713
- Brown TA, Nelson DE, Vogel JS, Southon JR. Improved collagen extraction by modified Longin method. Radiocarbon. 1988; 30: 171–177.
- 101. Balasse M, Ambrose SH, Smith AB, Price TD. The Seasonal Mobility Model for Prehistoric Herders in the South-western Cape of South Africa Assessed by Isotopic Analysis of Sheep Tooth Enamel. J Archaeol Sci. 2002; 29: 917–932.
- 102. Vaiglova P, Bogaard A, Collins M, Cavanagh W, Mee C, Renard J, et al. An integrated stable isotope study of plants and animals from Kouphovouno, southern Greece: A new look at Neolithic farming. J Archaeol Sci. 2014; 42: 201–215.
- 103. Nitsch EK, Charles M, Bogaard A. Calculating a statistically robust δ 13 C and δ 15 N offset for charred cereal and pulse seeds. Sci Technol Archaeol Res. 2015;1: STAR20152054892315Y.0000000001.
- 104. Wallace M, Jones G, Charles M, Fraser R, Halstead P, Heaton THE, et al. Stable carbon isotope analysis as a direct means of inferring crop water status and water management practices. World Archaeol. 2013; 45: 388–409.
- 105. Ambrose SH. Preparation and characterisation of bone and tooth collagen for isotopic analysis. J Archaeol Sci. 1990; 17: 431–451.

- **106.** DeNiro MJ. Post-mortem preservation and alteration of in vivo bone collagen isotope ratios on relation to palaeodietary reconstruction. Nature. 1985; 317: 806–809.
- 107. van Klinken GJ. Bone collagen quality indicators for palaeodietary and radiocarbon measurements. J Archaeol Sci. 1999; 26: 687–695.
- **108.** Nehlich O, Richards MP. Establishing collagen quality criteria for sulphur isotope analysis of archaeological bone collagen. Archaeol Anthropol Sci. 2009; 1: 59–75.
- Zazzo A. Bone and enamel carbonate diagenesis: A radiocarbon prospective. Palaeogeogr Palaeoclimatol Palaeoecol. 2014; 416: 168–178.
- 110. Zazzo A. Validation méthodologique de l'utilisation des compositions isotopiques (13C, 18O) des bioapatites fossiles pour la reconstitution des paléoenvironnements continentaux. Ph.D. Thesis, The Université Pierre et Marie Curie, Paris VI. 2001.
- 111. Koch PL, Tuross N, Fogel ML. The Effects of Sample Treatment and Diagenesis on the Isotopic Integrity of Carbonate in Biogenic Hydroxylapatite. J Archaeol Sci. 1997; 24: 417–429.
- 112. Zazzo A, Lécuyer C, Sheppard SMF, Grandjean P, Mariotti A. Diagenesis and the reconstruction of paleoenvironments: A method to restore original δ18O values of carbonate and phosphate from fossil tooth enamel. Geochim Cosmochim Acta. 2004; 68: 2245–2258.
- **113.** Wang Y, Cerling TE. A model of fossil tooth and bone diagenesis: implications for paleodiet reconstruction from stable isotopes. Palaeogeogr Palaeoclimatol Palaeoecol. 1994; 107: 281–289.
- 114. Styring AK, Charles M, Fantone F, Hald MM, McMahon A, Meadow RH, et al. Isotope evidence for agricultural extensification reveals how the world's first cities were fed. Nat Plants. 2017; 3: 17076. https://doi.org/10.1038/nplants.2017.76 PMID: 28581507
- 115. Fernandes R, Millard AR, Brabec M, Nadeau MJ, Grootes P. Food reconstruction using isotopic transferred signals (FRUITS): A bayesian model for diet reconstruction. PLoS One. 2014;9. <u>https://doi.org/ 10.1371/journal.pone.0087436 PMID: 24551057</u>
- 116. Hoefs J. Stable Isotope Geochemistry. Berlin: Springer; 2006.
- 117. Ivanov MV. The sulfur cycle in continental reservoirs. In: Ivanov MV, Freney JR, editors. The Global Biogeochemical Sulfur Cycle-Scope. John Wiley & Sons; 1983. pp. 297–356.
- **118.** Klee M, Brombacher C. Les donnees archaeobotaniques. Occupations protohistoriques a Chevenez: de l'Age du Bronze a l'Ahe du Fer. Cah d'Archaeologie jurassienne. 2010; 26: 154–158.
- 119. Signol C. Les macrorestes végétaux du site de Tougues à Chens-sur-Léman (Haute Savoie). MC.S. Thesis, The University of Franche-Comté, Besançon. 2000.
- 120. Cabanis M. Etude archaeobotanique. In: Néré E, editor. Chens-sur-Léman (Haute Savoie), rue de Charnage, Vérancy Nord: rapport de fouilles. Bron: INRAP; 2010. pp. 105–113.
- 121. Bouby L, Billaud Y. Identifying prehistoric collected wild plants: A case study from late bronze age settlements in the french alps (Grésine, Bourget Lake, Savoie). Econ Bot. 2005; 59: 255–267.
- 122. Cabanis M. Etude archaeobotanique. In: Néré E, editor. Chens-sur-Léman (Haute Savoie), rue du prè d'Ancy: rapport de fouilles Inrap. Bron: INRAP; 2014. pp. 181–191.
- 123. Varalli A, Grocke DR, Skeates R. Exploring dietary changes in the North Italian Metal Ages: a multiisotopic approach. Bull Mémoires la Société d'Anthropologie Paris. 2020; 32: S9–S33.
- 124. Häberle S, Fuller BT, Nehlich O, Van Neer W, Schibler J, Hüster Plogmann H. Inter- and intraspecies variability in stable isotope ratio values of archaeological freshwater fish remains from Switzerland (11th–19th centuries AD). Environ Archaeol. 2016; 21: 119–132.
- **125.** Masotti S, Varalli A, Goude G, Moggi-Cecchi J, Gualdi-Russo E. A combined analysis of dietary habits in the Bronze Age site of Ballabio (northern Italy). Archaeol Anthropol Sci. 2019; 11: 1029–1047.
- 126. Herrscher E, Goude G, Lisfranc R, Forest V, Vermeulen C. Pratiques alimentaires au Bronze ancien en Auvergne: Essai de restitution par l'analyse isotopique. Préhistoires Méditerranéennes. 2014;4.
- 127. Pokutta DA, Baron J, Dąbrowski P, Karlsson C. Bioarchaeology of Social Inequality in the Unetice Culture: A Case Study. In: Suchowska-Ducke P, Vandkilde H, Scott Reiter S, editors. Forging Identities The Mobility of Culture in Bronze Age Europe. Oxford: BAR International Series; 2015. pp. 111–119.
- 128. Knipper C, Pichler SL, Rissanen H, Stopp B, Kühn M, Spichtig N, et al. What is on the menu in a Celtic town? Iron Age diet reconstructed at Basel-Gasfabrik, Switzerland. Archaeol Anthropol Sci. 2017; 9: 1307–1326.
- 129. Tinner W, Lotter AF, Ammann B, Conedera M, Hubschmid P, van Leeuwen JFN, et al. Climatic change and contemporaneous land-use phases north and south of the Alps 2300 BC to 800 AD. Quat Sci Rev. 2003; 22: 1447–1460.
- **130.** Goude G, Rey L, Toulemonde F, Cervel M, Rottier S. Dietary changes and millet consumption in northern France at the end of Prehistory: Evidence from archaeobotanical and stable isotope data. Environ Archaeol. 2017; 22: 268–282.

- 131. Nehlich O, Wahl J. Binnengewässer—eine unterschätzte Nahrungsressource—Stabile Kohlenstoff-, Stickstoff- und Schwefelisotope aus dem Kollagen menschlicher und tierischer Knochenreste aus der urnenfelderzeitlichen Nekropole von Neckarsulm. Fundberichte aus Baden-württemb. 2011; 31: 97– 113.
- 132. Oelze VM, Koch JK, Kupke K, Nehlich O, Zäuner S, Wahl J, et al. Multi-isotopic analysis reveals individual mobility and diet at the Early Iron Age monumental tumulus of Magdalenenberg, Germany. Am J Phys Anthropol. 2012; 148: 406–21. https://doi.org/10.1002/ajpa.22063 PMID: 22553183
- **133.** Le Huray JD, Schutkowski H. Diet and social status during the La Tène period in Bohemia: Carbon and nitrogen stable isotope analysis of bone collagen from Kutná Hora-Karlov and Radovesice. J Anthropol Archaeol. 2005; 24: 135–147.
- 134. Gamarra B, Howcroft R, McCall A, Dani J, Hajdú Z, Nagy EG, et al. 5000 years of dietary variations of prehistoric farmers in the Great Hungarian Plain. PLoS One. 2018; 13: e0197214. <u>https://doi.org/10. 1371/journal.pone.0197214</u> PMID: 29746569
- 135. Murray ML, Schoeninger MJ. Diet, status, and complex social structure in Iron Age Central Europe: some contributions from bone chemistry. In: Gibson DBI, Geselowitz MN, editors. Tribe and Polity in Late Prehistoric Europe: Demography, Production and Exchange in the Evolution of Complex Social Systems. New York: Plenum Press; 1988. pp. 155–176.
- 136. Lightfoot E, Šlaus M, Šikanjić PR, O'Connell TC. Metals and millets: Bronze and Iron Age diet in inland and coastal Croatia seen through stable isotope analysis. Archaeol Anthropol Sci. 2015; 7: 375–386.
- 137. Varalli A, Sparacello VS, Dori I, Starnini E, Biagi P, Goude G. Ricostruzione dei pattern alimentari e delle pratiche di allattamento e svezzamento dal Neolitico alle età dei metalli tramite analisi multi-isotopiche della dentina incrementale e del collagene osseo. Riv di Sci Preist. 2020.
- Hatch JW, Geidel RA. Status-specific dietary variation in two world cultures. J Hum Evol. 1985; 14: 469–476.
- 139. Knipper C, Rihuete-Herrada C, Voltas J, Held P, Lull V, Micó R, et al. Reconstructing Bronze Age diets and farming strategies at the early Bronze Age sites of La Bastida and Gatas (southeast Iberia) using stable isotope analysis. PLoS One. 2020; 15: e0229398. <u>https://doi.org/10.1371/journal.pone.</u> 0229398 PMID: 32160202
- Triantaphyllou S, Richards MP, Zerner C, Voutsaki S. Isotopic dietary reconstruction of humans from Middle Bronze Age Lerna, Argolid, Greece. J Archaeol Sci. 2008; 35: 3028–3034.
- 141. Richards MP, Hedges REM. Stable isotope evidence of past human diet at the sites of the Neolithic cave of Gerani, the Late Minoan III cemetery of Armenoi, grave circles A and B at the palace site of Mycenae; and the Late Helladic Chamber Tombs. In: Tzedakis Y, Martlew H, Jones MK, editors. Archaeology Meets Science: Biomolecular Investigations in Bronze Age Greece. Oxford: Oxbow Books; 2008. pp. 220–230.
- 142. Knipper C, Meyer C, Jacobi F, Roth C, Fecher M, Stephan E, et al. Social differentiation and land use at an Early Iron Age "princely seat": bioarchaeological investigations at the Glauberg (Germany). J Archaeol Sci. 2014; 41: 818–835.
- 143. Beaumont J, Atkins EC, Buckberry J, Haydock H, Horne P, Howcroft R, et al. Comparing apples and oranges: Why infant bone collagen may not reflect dietary intake in the same way as dentine collagen. Am J Phys Anthropol. 2018; 167: 524–540. https://doi.org/10.1002/ajpa.23682 PMID: 30187451
- 144. Fuller BT, Fuller JL, Sage NE, Harris DA, O'Connell TC, Hedges REM. Nitrogen balance and δ15N: why you're not what you eat during nutritional stress. Rapid Commun Mass Spectrom. 2005; 19: 2497–506. https://doi.org/10.1002/rcm.2090 PMID: 16106342
- 145. Beaumont J, Montgomery J, Buckberry J, Jay M. Infant mortality and isotopic complexity: New approaches to stress, maternal health, and weaning. Am J Phys Anthropol. 2015; 157: 441–457. https://doi.org/10.1002/ajpa.22736 PMID: 25773670
- 146. Henderson RC, Lee-Thorp J, Loe L. Early life histories of the London poor using δ13C and δ15N stable isotope incremental dentine sampling. Am J Phys Anthropol. 2014; 154: 585–93. <u>https://doi.org/10.1002/ajpa.22554 PMID: 24898314</u>
- 147. D'Ortenzio L, Brickley M, Schwarcz H, Prowse T. You are not what you eat during physiological stress: Isotopic evaluation of human hair. Am J Phys Anthropol. 2015;157.
- 148. Neuberger FM, Jopp E, Graw M, Püschel K, Grupe G. Signs of malnutrition and starvation-Reconstruction of nutritional life histories by serial isotopic analyses of hair. Forensic Sci Int. 2013; 226: 22– 32. https://doi.org/10.1016/j.forsciint.2012.10.037 PMID: 23374882
- 149. Goude G, Dori I, Sparacello VS, Starnini E, Varalli A. Multi-proxy dentine microsections analyses reveal diachronic changes in life history adaptations, mobility, and tuberculosis-induced wasting in prehistoric Liguria (Finale Ligure, Italy, northwestern Mediterranean). Int J Paleopathol. 2020; 28: 99– 111. https://doi.org/10.1016/j.ijpp.2019.12.007 PMID: 32044292

- Walter BS, DeWitte SN, Dupras T, Beaumont J. Assessment of nutritional stress in famine burials using stable isotope analysis. Am J Phys Anthropol. 2020; 172: 214–226. <u>https://doi.org/10.1002/ajpa.</u> 24054 PMID: 32243588
- Beaumont J, Gledhill A, Lee-Thorp J, Montgomery J. Childhood diet: A closer examination of the evidence from dental tissues using stable isotope analysis of incremental human dentine. Archaeometry. 2013; 55: 277–295.
- 152. Beaumont J, Montgomery J. The Great Irish Famine: Identifying Starvation in the Tissues of Victims Using Stable Isotope Analysis of Bone and Incremental Dentine Collagen. PLoS One. 2016; 11: e0160065. https://doi.org/10.1371/journal.pone.0160065 PMID: 27508412
- Katzenberg MA, Lovell NC. Stable isotope variation in pathological bone. Int J Osteoarchaeol. 1999; 9: 316–324.
- 154. Sandberg PA, Sponheimer M, Lee-Thorp J, Van Gerven D. Intra-tooth stable isotope analysis of dentine: A step toward addressing selective mortality in the reconstruction of life history in the archaeological record. Am J Phys Anthropol. 2014; 155: 281–293. <u>https://doi.org/10.1002/ajpa.22600</u> PMID: 25156177
- 155. Prowse T, Schwarcz HP, Saunders S, Macchiarelli R, Bondioli L. Isotopic paleodiet studies of skeletons from the imperial Roman-age cemetery of Isola Sacra. J Archaeol Sci. 2004; 31: 259–272.
- 156. Varalli A, Desideri J, Goude G, David-Elbiali M, Besse M. Should I stay or should I go? Assessing residential mobility in Bronze Age Switzerland through the sulphur and strontium isotopic evidence. Am J Phys Anthropol. 2019; 168: 255–256.
- 157. Ambrose SH, Buikstra J, Krueger HW. Status and gender differences in diet at Mound 72, Cahokia, revealed by isotopic analysis of bone. J Anthropol Archaeol. 2003; 22: 217–226.
- **158.** Hedges REM. On bone collagen-apatite-carbonate isotopic relationships. Int J Osteoarchaeol. 2003; 13: 66–79.
- 159. France CAM, Owsley DW. Stable Carbon and Oxygen Isotope Spacing Between Bone and Tooth Collagen and Hydroxyapatite in Human Archaeological Remains. Int J Osteoarchaeol. 2015; 25: 299–312.
- Froehle a W, Kellner CM, Schoeninger MJ. Multivariate carbon and nitrogen stable isotope model for the reconstruction of prehistoric human diet. Am J Phys Anthropol. 2012; 147: 352–69. https://doi.org/ 10.1002/ajpa.21651 PMID: 22213005
- Schwarcz HP. Some theorical aspects of isotope paleodiet studies. J Archaeol Sci. 1991; 18: 261– 275.
- 162. Zhu M, Sealy J. Multi-tissue stable carbon and nitrogen isotope models for dietary reconstruction: Evaluation using a southern African farming population. Am J Phys Anthropol. 2018; 168: 145–153. https://doi.org/10.1002/ajpa.23731 PMID: 30379328
- 163. France CAM, Owsley DW. Stable Carbon and Oxygen Isotope Spacing Between Bone and Tooth Collagen and Hydroxyapatite in Human Archaeological Remains. Int J Osteoarchaeol. 2015; 25: 299–312. https://doi.org/10.1002/oa.2300
- 164. Santana-Sagredo F, Lee-Thorp JA, Schulting R, Uribe M. Isotopic evidence for divergent diets and mobility patterns in the Atacama Desert, Northern Chile, During the Late Intermediate Period (AD 900–1450). Am J Phys Anthropol. 2015; 156: 374–387. https://doi.org/10.1002/ajpa.22663 PMID: 25385676
- 165. Becdelièvre C. Ecology and Ethology of the Danube Gorges population ca 9500–5500 BC Bioarchaeological perspectives on dietary behaviors and adaptive strategies during the Mesolithic and Neolithic Transformations. Ph.D. Thesis, The University of Belrgrade. 2020.