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Comparative regional stable isotope analysis reveals fluctuations in millet consumption during the Bronze and Iron Ages in the Eastern Adriatic

Valentina Martinoia[1]✉, Mario Carić[2], Boris Čargo[3], Martina Čelhar[4], Natalija Čondić[5], Slavko Galiot[6], Domagoj Perkić[7], Dinko Tresić Pavičić[8], Marina Ugarković Džafić[9], Morana Vuković[5], Joško Zaninović[10], Urszula Zientek-Androulaki[11], Emily Zavodny[12], Sarah McClure[13] & Mario Novak[2,14]

This study presents the largest regional-scale stable isotope investigation of human and faunal remains spanning the Bronze and Iron Ages (*c.* 2400–100 BCE) in the Eastern Adriatic to date. New $\delta^{13}C$ and $\delta^{15}N$ data from 51 individuals were integrated with previously published results (n = 233) to explore long-term and region-specific dietary trends, with a focus on the emergence of millet as a dietary component. Results reveal an inland–coastal contrast: while coastal populations predominantly consumed C_3-based diets, inland groups show consistently higher $\delta^{13}C$ values indicative of direct millet intake from the Middle/Late Bronze Age onwards. Statistical analyses confirm strong effects of both period and location on $\delta^{13}C$ values, with most inland populations exhibiting more positive signatures particularly during the Middle/Late Bronze Age transition and again in the Iron Age. A probabilistic two-endmember mixing model estimates millet contributions reaching up to ~40% of dietary protein in these inland groups, suggesting episodic but significant integration of C_4 crops into subsistence systems. In contrast, faunal baselines reflect purely C_3 diets, indicating that the C_4 signal in humans derives from direct millet consumption. These findings help refine the chronology of millet adoption in the eastern Adriatic, pushing its significant dietary incorporation back to the Middle/Late Bronze Age transition, and highlight fluctuating patterns of C_4 plant consumption through the Bronze and Iron Ages, likely shaped by ecological conditions and local adaptive strategies rather than uniform cultural change.

Keywords Eastern Adriatic, Bronze Age, Iron Age, Millet, Stable isotope analysis

The Bronze (*c.* 2400–1000/900 BCE) and Iron Ages (*c.* 1000/900–100 BCE) in the Eastern Adriatic represent dynamic periods of cultural, economic, and subsistence change, shaped both by long-distance connections across the Adriatic and the Carpathian Basin, as well as local responses to shifting environmental and social conditions[1,2]. Inland Croatia, part of the Carpathian Basin, lay within one of the principal centres of Bronze Age innovation[3], while the Dalmatian coast became increasingly important as maritime routes linked the region to

[1]Department of Medicine, Section of Human Anatomy, University of Udine, 33100 Udine, Italy. [2]Center for Applied Bioanthropology, Institute for Anthropological Research, 10000 Zagreb, Croatia. [3]Archaeological Museum in Split, 21000 Split, Croatia. [4]Department of Archaeology, University of Zadar, 23000 Zadar, Croatia. [5]Archaeological Museum Zadar, 23000 Zadar, Croatia. [6]Delmat Galiot Ltd., 21000 Split, Croatia. [7]Archaeological Museum, Dubrovnik Museums, 20000 Dubrovnik, Croatia. [8]Kaducej Ltd, 21000 Split, Croatia. [9]Institute of Archaeology, 10000 Zagreb, Croatia. [10]Krka National Park, 22000 Šibenik, Croatia. [11]Independent Researcher, 70007 Malia, Crete, Greece. [12]Department of Anthropology, University of Central Florida, Orlando, FL 32816, USA. [13]Department of Anthropology, University of California, Santa Barbara, CA 93106-3210, USA. [14]Department of Archaeology and Heritage, Faculty of Humanities, University of Primorska, 6000 Koper, Slovenia. ✉email: valentina.martinoia@uniud.it

Italy and the Aegean[4,5]. Additionally, advances in metallurgy[5] and control over metal resources underpinned the rise of social elites and contributed to growing social stratification[6–8].

Subsistence strategies also changed significantly, shaped by cultural innovations and climatic conditions. Culturally, the introduction of the horse from eastern steppe regions into the Carpathian Basin during the Late Copper Age (*c.* 3500–2500 BCE), and its subsequent spread during the Bronze Age, transformed mobility, communication, and agricultural labour[9,10]. The adoption of the plough[11] further facilitated more extensive cultivation, reducing the effort required to prepare fields and allowing larger tracts of land to be exploited. Climatically, the entirety of the Bronze Age and the beginning of the Iron Age in this region coincided with the Sub-Boreal period (*c.* 3300–800 BC), which was generally cool and wet[12]. Reconstructions suggest that average temperatures were only 1–2 °C lower than today[13], yet the transition from the preceding warm and moist Atlantic period (*c.* 5500–3000 BC)[14] likely had significant consequences for agriculture. Even modest cooling and increased rainfall may have required adjustments in crop selection and farming strategies, influencing the resilience and adaptability of Bronze Age agricultural systems.

Archaeobotanical evidence from Bronze Age Dalmatia indicates that core C_3 domesticates such as emmer (*Triticum dicoccum*), einkorn (*Triticum monococcum*), wheat (*Triticum aestivum*), and barley (*Hordeum vulgare*) were central to subsistence in both coastal and inland Croatian contexts, while broomcorn (*Panicum miliaceum*) and, to a lesser extent, foxtail (*Setaria italica*) millet were the only staple C_4 crops[5,15–20].

Introduced to the Eastern Adriatic by the mid-fifteenth century BCE, based on radiocarbon and archaeobotanical evidence[21], millet was initially marginal but its adoption was already widespread by the end of the Bronze Age in several areas of Europe[22–25], likely due to its short growing cycle, low labour requirements, and resilience under variable climatic and environmental conditions[18]. In addition, it offers substantial nutritional benefits, with protein, vitamin, and mineral contents often exceeding those of more common cereals such as wheat[26]. Given these properties, millet would have represented a risk-averse and adaptable option, particularly under the cooler and wetter conditions of the Sub-Boreal climatic period.

During the Iron Age, agriculture in the region diversified further. On the Dalmatian coast, archaeobotanical finds document the cultivation of spelt wheat (*Triticum spelta*), six-row barley (*Hordeum hexastichum*), broomcorn millet (and possibly foxtail millet), along with grape and several gathered plants[18]. Comparative evidence from inland sites in neighbouring Slovenia and the Danube Basin points to a wide range of cereals, including einkorn, emmer, spelt, barley, oats (*Avena sativa*), rye (*Secale cereale*), and millet, alongside pulses[18].

By the end of the Iron Age, the integration of broomcorn and foxtail millet into agricultural systems varied considerably. In regions such as Slovenia[27], Croatia[28], and northern Italy and the Eastern Alps[29–34], millet continued to be cultivated into the Roman period, whereas in central and western Europe[35,36] its use declined sharply. These divergent patterns suggest that decisions to adopt or abandon millet were influenced by local environmental conditions, established farming traditions, and the technological practices of different communities[37].

Zooarchaeological data complement these findings. Bronze Age assemblages in the Carpathian Basin and eastern Adriatic demonstrate mixed economies in which domesticated livestock were exploited not only for meat but also for secondary products such as milk and wool[38,39], supplemented by hunting of deer, hare, and other wild taxa[40–42]. Iron Age sites on the Dalmatian coast show a strong emphasis on sheep (*Ovis aries*) and goats (*Capra hircus*), alongside cattle (*Bos taurus*), domesticated (*Sus domesticus*) and wild (*Sus scrofa*) pigs, fowl, and a range of wild animals, suggesting that ovicaprids played a central role in subsistence[15,43]. Although faunal evidence from inland Iron Age Croatia is scarce, available data[40,44] and comparative material from Bosnia and Hungary[42,45] point to herding economies focused on cattle, sheep/goats, pigs, horses (*Equus caballus*), and poultry, with wild resources such as deer (*Cervus elaphus*), fish, and waterfowl contributing to diets. Kill-off profiles indicate that pigs were primarily raised for meat, sheep and goats for wool and milk, and cattle for both primary and secondary products[18].

Previous isotopic research in the Eastern Adriatic

In the Eastern Adriatic, particularly in inland areas where archaeobotanical and zooarchaeological evidence remains patchy, stable isotope analysis offers an essential line of evidence for understanding long-term dietary change. Previous isotopic research[28] on 67 Iron Age individuals from Dalmatia indicated no evidence for seafood consumption and diets based predominantly on C_3 plants, although a few outliers suggest substantial millet intake. Subsequent research conducted by Lightfoot and colleagues[18] on Bronze and Iron Age populations from coastal and inland Croatia, based on 50 bone samples from 10 sites, indicates that C_4 plants contributed little to diet during the Bronze Age. Coastal individuals show only a slight increase in millet consumption at the Bronze–Iron Age transition, whereas inland individuals exhibit substantial millet intake during the Iron Age. These patterns align with archaeobotanical[17,19] and radiocarbon[21] evidence suggesting that millet became consistently integrated into human diets in Croatia from the Late Bronze Age onward. Notably, the authors suggest that despite the limited faunal baseline, animal remains provide no evidence of C_4 plant consumption in either period, supporting the interpretation that the C_4 signal in humans reflects direct millet consumption rather than indirect incorporation through animal protein. This pattern is reinforced by isotopic evidence from Bronze Age Lika (inland Croatia), where Zavodny et al.[40] demonstrated that domesticated livestock exhibit $\delta^{13}C$ values indistinguishable from those of wild fauna, with only a few exceptions among pigs.

Zavodny and colleagues[37] also reported that individuals from 12 Bronze and Iron Age sites in and around the Lika region consumed notable amounts of C_4 plants, in some cases up to ~20% of dietary protein, with the earliest evidence detectable as far back as the Middle Bronze Age. They further argued that millet cultivation may have served as an adaptive response to climatic instability. According to their findings, millet consumption in Lika persisted into later periods and, in certain Iron Age contexts, may have reached contributions of up to ~40% of the diet.

This pattern is further supported by data from Martinoia et al.[46] who reported significant C_4 input in the childhood diets of 16 individuals from Bezdanjača Cave (Lika). These findings suggest a greater emphasis on millet consumption in inland areas of Croatia during the Late Bronze and Iron Ages, consistent with previously reported patterns[18,37].

Lastly, recent isotopic analysis of 28 individuals from the Iron Age coastal site of Nadin–Gradina in Dalmatia[47] revealed a relatively homogeneous diet dominated by C_3 plant resources, with only minor and sporadic examples of significant contribution from C_4 plants. In contrast, contemporary inland sites in Croatia and Slovenia show a stronger reliance on C_4 plants and lower $\delta^{15}N$ values.

Building on this background, we present a regional comparative analysis of new and previously published $\delta^{13}C$ and $\delta^{15}N$ data from Bronze and Iron Age communities across the Eastern Adriatic. Our objectives are threefold: (1) to evaluate the relative contributions of plant versus animal protein to human subsistence strategies, (2) to assess the timing and extent of millet consumption and its integration into local diets, and (3) to examine potential dietary differences between inland and coastal populations through the Bronze and Iron Ages.

Results

This study presents new isotopic data from 51 human and 3 faunal bone samples recovered from 23 sites in present-day Croatia ($n_{sites} = 22$) and Montenegro ($n_{sites} = 1$). The human individuals are distributed across the Bronze Age ($n_{humans} = 20$), the Bronze/Iron Age transition ($n_{humans} = 5$), and the Iron Age ($n_{humans} = 26$). Of these, 43 individuals derive from coastal sites and 8 from inland sites (Fig. 1). The distinction between "coastal" and "inland" sites is used to explore spatial variation in dietary patterns and was defined based on both the distance of each site from the present-day coastline and its environmental setting. "Coastal" sites include those located on or within the coastal littoral, in close proximity to the shoreline, whereas "inland" sites are conservatively defined as those situated beyond the immediate coastal zone, typically more than 40 km from the present-day coastline, and characterized by hinterland environmental conditions.

Although relative sea level during the Bronze and Iron Ages differed from present values (generally by only a few meters in both the Mediterranean and Adriatic basins[48,49]), such changes would have affected the precise position of the shoreline but are unlikely to have altered the broader distinction between coastal and hinterland zones at the regional scale considered here. Accordingly, the present-day coastline is used as a consistent proxy for approximating past spatial relationships, and the coastal–inland classification adopted here likely reflects, in broad terms, relative distance from the coastline in the past.

On this basis, only three sites (Jazinka Cave, Tuzi—Planinica, and Rudine–Izvor Cetine) are classified as inland, while all others (n = 20) are considered coastal or part of the coastal hinterland.

Archaeological information, site chronologies and, when available, associated radiocarbon dates are provided in Supplementary Information S1.

Three newly analyzed faunal samples from the MBA/LBA site of Obličevac–Gomila (Neretva region) were added to the dataset: one cattle [*Bos taurus*] ($\delta^{13}C = -20.6‰$, $\delta^{15}N = 4.8‰$), one marmot [*Marmota marmota*] ($\delta^{13}C = -20.1‰$, $\delta^{15}N = 4.3‰$), and one ovicaprid [*Ovis/Capra*] ($\delta^{13}C = -20.4‰$, $\delta^{15}N = 4.9‰$). Their values are consistent with a C_3 terrestrial baseline and fall within the expected range for herbivorous and predominantly C_3-feeding species. Given the limited number of newly analyzed faunal samples, and the absence of site-specific faunal data for many of the locations included in this study, these data are not used to establish an independent isotopic baseline. Instead, they are integrated with previously published faunal datasets from Croatia[28,40] (Supplementary Information S2) to construct a broader comparative framework.

The combined faunal dataset is geographically and chronologically constrained, spanning the Middle and Late Bronze Age and Iron Age, and is largely derived from inland contexts. As such, it may not fully capture regional or environmental variability, and some variation in human isotopic values may reflect environmental differences rather than dietary variation alone. Nonetheless, it represents the most comprehensive baseline currently available for the region and period. The resulting faunal baseline therefore comprises 62 individuals, including both domesticated (cattle [n = 18], ovicaprids [n = 15], horses [n = 2]) and wild herbivores (deer [n = 7], a hare, and a marmot), as well as domesticated omnivores (pigs [n = 10] and dogs [n = 8]).

Overall, the average $\delta^{13}C$ value for herbivores is $-20.8 \pm 1.0‰$ (min: −22.9‰; max: −16.2‰), while their average $\delta^{15}N$ value is $5.0 \pm 1.0‰$ (min: 2.8‰; max: 7.9‰). For the omnivores, the average $\delta^{13}C$ value is $-19.2 \pm 1.1‰$ (min: −21.1‰; max: −17.3‰) and their average $\delta^{15}N$ value is $6.8 \pm 1.5‰$ (min: 3.1‰; max: 10.2‰).

Exploratory comparisons of faunal isotope values across chronological groups indicate some diachronic variation in herbivore $\delta^{13}C$ values (Kruskal–Wallis, $p = 0.0022$), while $\delta^{15}N$ values show less of a difference ($p = 0.184$). Conversely, omnivore values show little variation through time for either $\delta^{13}C$ ($p = 0.34$) or $\delta^{15}N$ ($p = 0.28$). A Wilcoxon rank-sum test revealed a significant difference in $\delta^{13}C$ values between inland and coastal herbivores ($W = 282$, $p = 0.0003$), indicating spatial variation in baseline carbon isotope values. While this variation is not unexpected given the differences in climate and environment (e.g., forest cover, aridity) between each region, values are still consistent with C_3-dominated landscapes. In contrast, $\delta^{15}N$ values show more limited variation between locations ($p = 0.68$). Similarly, omnivore $\delta^{13}C$ and $\delta^{15}N$ values show little spatial variation ($p > 0.24$).

The isotopic results for the 51 human individuals analyzed in this study (Supplementary Information S2) are summarized in Table 2 and illustrated in Fig. 2. Of the human individuals, 35 were classified as adults and 13 as nonadults (Table 1), with the age threshold set at 17 years following established osteological criteria[50,51].

Overall, the mean $\delta^{13}C$ values for the humans are $-19.2 \pm 0.8‰$ (adults: $-19.3 \pm 0.8‰$, nonadults: $-18.7 \pm 0.9‰$), while the mean $\delta^{15}N$ values are $9.4 \pm 1.0‰$ (adults: $9.3 \pm 0.9‰$, nonadults: $9.6 \pm 1.5‰$). Although a two-sample t-test indicates a statistically significant difference in $\delta^{13}C$ values between adults and nonadults ($p = 0.020$), the isotopic offset is small (< 1‰) and no significant difference is observed in $\delta^{15}N$ values ($p = 0.413$).

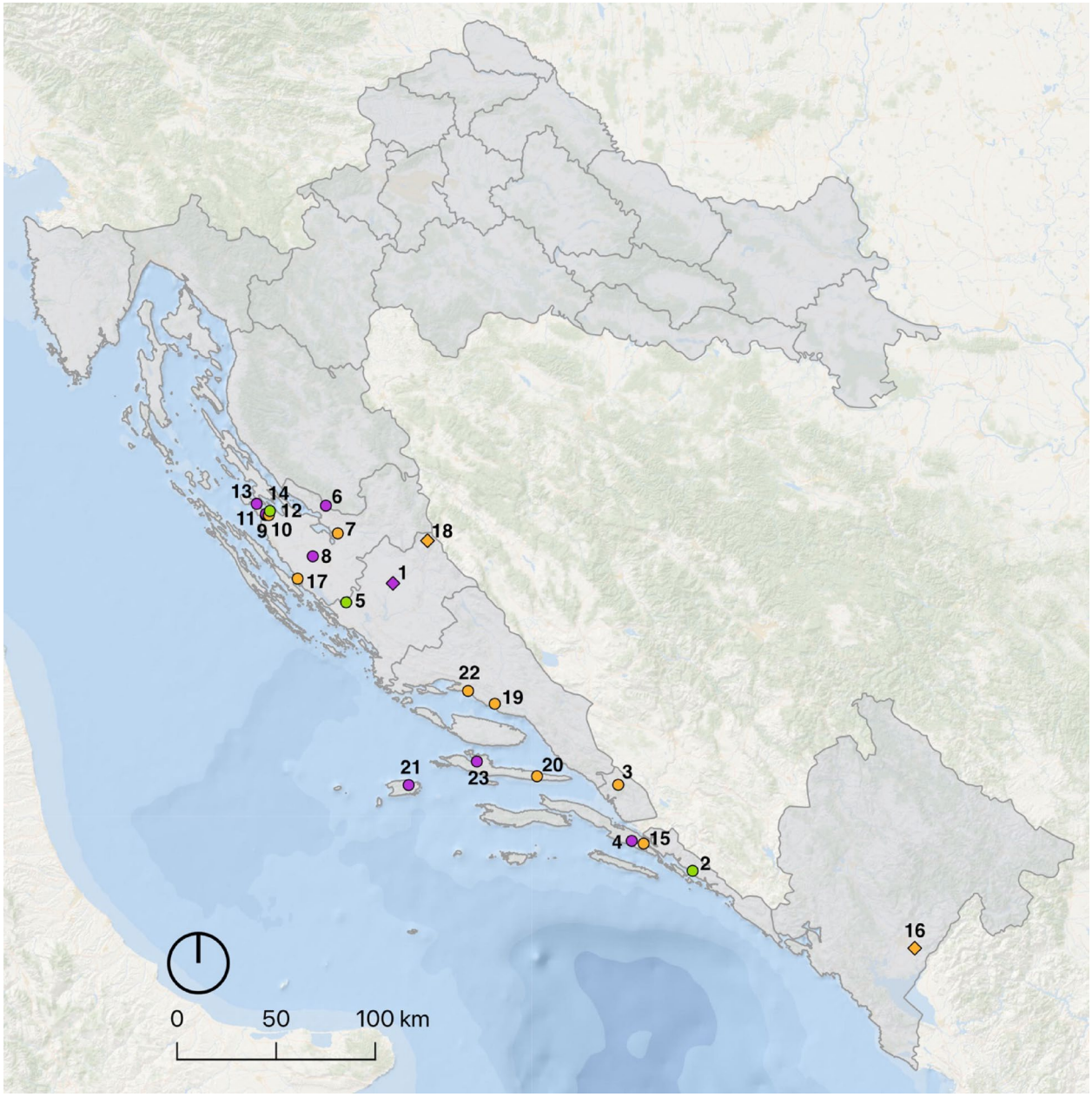

Fig. 1. Location of the samples analyzed in this study. Sites are categorized as coastal (dots) or inland (diamonds). Colors indicate chronology: orange: Bronze Age; green: Bronze/Iron Age; purple: Iron Age. Numbered sites are as follows: 1: Jazinka Cave; 2: Kukova peć Cave; 3: Obličevac—Gomila; 4: Zabrđe—Popov dol; 5: Stankovci—Velim; 6: Malo Libinje—Gradina Kneževići; 7: Kruševo—Vjetroelektrane; 8: Nadin; 9: Nin; 10: Nin—Solana; 11: Nin—Ždrijac; 12: Nin—Rašnovac; 13: Vir—Virić; 14: Vrsi—Mulo; 15: Gudnja Cave; 16: Tuzi—Planinica; 17: Turanj—Gradina; 18: Rudine–Izvor Cetine; 19: Donja Ostrvica—Pasičine; 20: Hvar—Bogomolje; 21: Vis Mijurovac; 22: Stobreč; 23: Hvar—Stari Grad.

As shown in Table 1, the nonadult group includes individuals at different developmental stages, including several prenatal individuals, one 3–4-week-old infant, and adolescents. Previous studies[52] have shown that isotopic enrichment related to breastfeeding may not be clearly expressed in bone collagen during the first three months of life, while older individuals would no longer be expected to exhibit such enrichment. Although a breastfeeding effect cannot be excluded for individuals broadly classified as "nonadults," the absence of a significant difference in $\delta^{15}N$ values between adults and nonadults, together with the statistical results, supports the interpretation that the nonadult sample does not show a clear breastfeeding signal at the group level[53,54]. On this basis, adults and nonadults are considered together in the following analyses (Table 2).

To assess variation across time periods, a one-way ANOVA with post hoc Tukey HSD tests was employed (Supplementary Information S3). The analysis revealed a significant effect of time period on $\delta^{13}C$ values ($F = 3.993$, $p = 0.000783$), whereas $\delta^{15}N$ values show less variation across chronological periods ($F = 1.617$, $p = 0.137$). Post

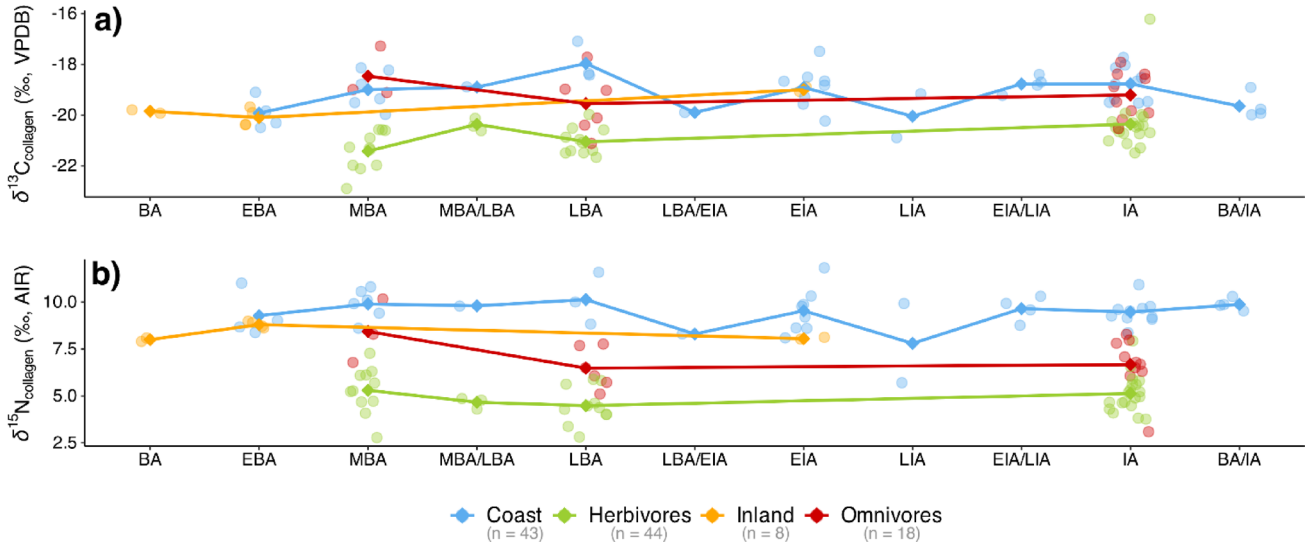

Fig. 2. (**a**) $\delta^{13}C$ and (**b**) $\delta^{15}N$ isotope results for the 51 humans analyzed in this study, divided by location (coast vs. inland sites) and period. Baseline values for herbivores and omnivores ($n_{total} = 62$) include newly generated samples (n = 3) and previously published data (n = 59)[28,40]. Solid diamonds represent mean values per period and location.

Site	Sample ID	Period	Age
Zabrđe—Popov dol	Mound	Late Iron Age	Nonadult
Malo Libinje—Gradina Knežević	Grave 3	Iron Age	Nonadult
Nin	Grave 58	Early Iron Age	12–18 years old
Nin—Solana	Grave 69	Early/Late Iron Age	Prenatal
	Grave 62		Prenatal
	Grave 74		Prenatal
Vrsi—Mulo	Mound 8, Grave 4, individual A	Early Iron Age	5–6 years old
	Mound 8, Grave 4, individual C		Adolescent
Gudnja Cave	Individual 1	Middle Bronze Age	3–4 weeks
	Individual 2		Prenatal
	Individual 3		Prenatal
Turanj—Gradina	G 2	Middle Bronze Age	Nonadult
Stobreć	SJ 293	Late Bronze Age	Nonadult

Table 1. Nonadult individuals included in this study. When available, precise age-at-death estimates are reported. The individual from Vrsi–Mulo classified as "adolescent" was included in the nonadult group due to the lack of a more precise age estimate.

hoc Tukey tests indicate that $\delta^{13}C$ differences are not uniformly distributed through time and are primarily driven by contrasts involving the EBA. In particular, individuals dating to the EBA exhibit significantly more negative $\delta^{13}C$ values compared to those from the LBA ($p = 0.0017$), EIA ($p = 0.040$), and IA ($p = 0.010$), with the largest offset observed between the EBA and LBA (≈ 2.0‰). A further significant difference is observed between the LBA and LIA ($p = 0.040$), indicating additional variability in $\delta^{13}C$ values in later periods. In contrast, $\delta^{13}C$ values among periods later than the EBA show more limited differentiation, with most pairwise comparisons not reaching statistical significance after correction for multiple testing, suggesting substantial overlap between groups.

In terms of location, coastal individuals have mean $\delta^{13}C$ values of −19.0 ± 0.8‰ (min −20.9‰, max −17.1‰) and mean $\delta^{15}N$ values of 9.5 ± 1.0‰ (min 5.7‰, max 11.8‰) (Table 2). Inland individuals show similar $\delta^{13}C$ values (−19.8 ± 0.5‰; min −20.4‰, max −18.9‰) and slightly lower $\delta^{15}N$ values (8.4 ± 0.4‰; min 7.9‰, max 9.0‰) than coastal individuals. While the offsets in both $\delta^{13}C$ and $\delta^{15}N$ means between coastal and inland groups are modest (≈ 1‰), statistically significant differences are observed in both $\delta^{13}C$ and $\delta^{15}N$ values (Wilcoxon rank-sum test: $\delta^{13}C$, $p = 0.017$; $\delta^{15}N$, $p = 0.00082$). These results should, however, be interpreted with caution given the limited number of inland individuals in the dataset, which affect the robustness of spatial comparisons.

	n	Mean $\delta^{13}C$	SD $\delta^{13}C$	Mean $\delta^{15}N$	SD $\delta^{15}N$
Period					
BA (*c.* 2400–1000/900 BCE)	2	−19.8	0.1	8.0	0.1
EBA (*c.* 2400–1600 BCE)	8	−20.0	0.5	9.0	0.8
MBA (*c.* 1600–1200 BCE)	6	−19.0	1.0	9.9	0.8
MBA/LBA (*c.* 1200 BCE)	1	−18.9	–	9.8	–
LBA (*c.* 1200–900 BCE)	3	−17.9	0.8	10.1	1.4
LBA/EIA (*c.* 1000–900 BCE)	1	−19.9	–	8.3	–
EIA (*c.* 1000/900–600 BCE)	10	−18.9	0.7	9.2	1.2
LIA (*c.* 700/600–100 BCE)	2	−20.0	1.0	7.8	3.0
EIA/LIA (*c.* 1000/900–100 BCE)	4	−18.8	0.3	9.7	0.6
IA (*c.* 1000/900–100 BCE)	10	−18.8	0.7	9.5	0.6
BA/IA (*c.* 2400–100 BCE)	4	−19.6	0.5	9.9	0.3
Location					
Coastal	43	−19.0	0.8	9.5	1.0
Inland	8	−19.8	0.5	8.4	0.4

Table 2. $\delta^{13}C$ and $\delta^{15}N$ mean and standard deviation for the samples analyzed in this study, grouped by period (BA = Bronze Age; EBA = Early Bronze Age; MBA = Middle Bronze Age; MBA/LBA = Middle/Late Bronze Age; LBA = Late Bronze Age; EIA = Early Iron Age; LIA = Late Iron Age, EIA/LIA = Early/Late Iron Age; IA = Iron Age; BA/IA = Bronze/Iron Age) and location (coastal vs inland). All values are expressed in ‰.

Discussion

Our new results indicate both distinct temporal and spatial variability in isotopic data, although these patterns must be interpreted in light of the uneven distribution of samples across chronological phases and regions. Inland individuals are few and limited to a small number of periods (BA [sub-phase unspecified due to limited chronological resolution], EBA, and EIA), with the highest $\delta^{13}C$ values observed in the EIA; however, the restricted sample size precludes the identification of clear diachronic trends in inland contexts based on the newly generated data alone.

By contrast, coastal individuals display a more structured pattern, with $\delta^{13}C$ values gradually increasing from the MBA and reaching their highest values in the LBA, followed by a marked decrease at the LBA/EIA transition. Thereafter, $\delta^{13}C$ values fluctuate considerably. However, some phases (e.g., MBA/LBA, LBA, LBA/EIA, LIA, and EIA/LIA) are represented by few individuals, and the magnitude of these changes therefore warrants cautious interpretation.

Faunal data provide a critical framework for interpreting these patterns. Statistical analyses have shown that herbivore $\delta^{13}C$ values differ between coastal and inland environments, indicating that part of the spatial variation in human $\delta^{13}C$ reflects underlying environmental differences in vegetation and baseline carbon isotope values. In contrast, herbivore $\delta^{15}N$ values do not vary significantly between locations, whereas human $\delta^{15}N$ values are significantly higher in coastal populations. This mismatch suggests that the observed $\delta^{15}N$ differences in humans are not primarily driven by baseline variation but instead reflect differences in dietary practices, though this interpretation should be treated with caution given the considerable imbalance in sample size between coastal and inland individuals. Nonetheless, the absence of $\delta^{13}C$ values consistent with marine resource consumption argues against significant marine fish intake in coastal populations, although the specific protein sources responsible for the elevated coastal $\delta^{15}N$ values cannot be determined from the current data alone.

Across all periods, herbivore $\delta^{13}C$ and $\delta^{15}N$ values remain consistent with a C_3-based terrestrial ecosystem, with no evidence for C_4 plant consumption. This supports the interpretation that elevated $\delta^{13}C$ values observed in humans are unlikely to derive from animal protein and instead reflect the direct consumption of C_4 resources, such as millet. Omnivore values partially overlap with those of humans but do not display consistent temporal or spatial trends, likely reflecting opportunistic feeding behavior or close human management[55] rather than structured dietary patterns.

Given that not all chronological phases and locations are equally represented in the newly analyzed sample, the isotopic data generated in this study were combined with previously published datasets[18,28,37,40,46,47,56] from Croatia ($n_{humans} = 233$), producing a comparative framework of 284 humans and 62 faunal remains (Table 3) spanning from the EBA to the LIA (Supplementary Information S2). As such, the diachronic patterns discussed below are best understood as emerging from this combined dataset rather than from the newly generated samples alone.

To identify broad temporal patterns, individuals were grouped into three major categories (Bronze Age, Bronze/Iron Age transition, and Iron Age). As illustrated in Figs. 3 and 4, most inland individuals from both the Bronze and Iron Ages exhibit relatively higher $\delta^{13}C$ values, suggesting the incorporation of C_4 plants such as millet into their diets. Nitrogen values for the human sample range between 4.8 and 13.3‰, suggesting varied protein sources derived primarily from terrestrial herbivores and omnivores.

Two exceptions are noteworthy: one BA individual from this study (POPAN43UI) and one IA individual (ELFL53 from Lightfoot et al.[18,28]), both coastal, with $\delta^{15}N$ values < 6‰ and plotting alongside herbivores. These values may reflect low to no animal protein intake, potentially indicating diets in which protein was derived

Group	Location	Period	n
Herbivores	Coast	BA	3
	Inland		22
	Coast	IA	6
	Inland		13
Omnivores	Coast	BA	–
	Inland		8
	Coast	IA	4
	Inland		6

Table 3. Number of herbivores and omnivores considered in this study, by period and location.

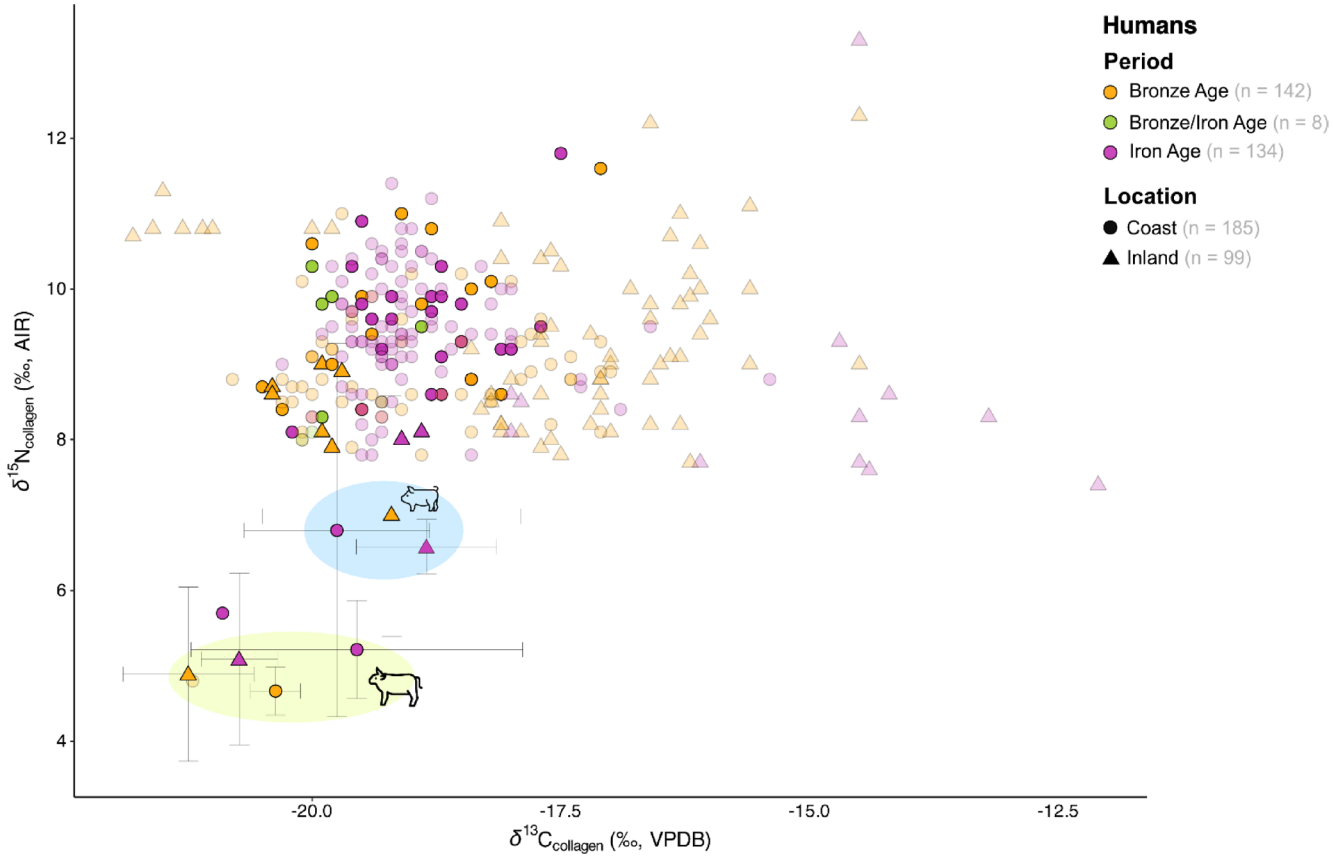

Fig. 3. Scatterplot of $\delta^{13}C$ and $\delta^{15}N$ values of human and faunal samples from the Bronze and Iron Ages. Data generated in this study are shown as solid symbols, while previously published data[18,28,37,40,46,47,56] are shown with reduced transparency (except for faunal values). Herbivores and omnivores are highlighted by yellow and blue circles, respectively. See Table 3 for the number of herbivore and omnivore samples by period and location.

primarily from plant-based resources, including legumes, which can contribute to lower $\delta^{15}N$ values due to their nitrogen-fixing properties[57]. Alternatively, consumption of herbivorous waterfowl feeding on algae or seagrass could produce similarly low $\delta^{15}N$ values without implying reduced animal protein intake overall.

In contrast, six Bronze Age individuals from Vučedol[56] exhibit the lowest $\delta^{13}C$ values of the dataset, consistent with a wholly C_3-based diet and little or no contribution from fish, despite proximity to the Danube. A small subset of IA coastal individuals[28] stand out as outliers with higher $\delta^{13}C$. As noted in the original publication[28], the values of these coastal individuals likely reflect the consumption of significant quantities of millet rather than marine resources, possibly indicating localized or individual-level dietary variation within otherwise predominantly C_3-based diet. Their presence suggests that millet use was not entirely absent in coastal settings but rather occurred sporadically and did not represent a consistent or widespread dietary pattern.

Overall, no population appears to have relied significantly on marine or freshwater resources (with the possible exception of individuals BzV-10 from Bezdanjača [MBA/LBA; $\delta^{13}C$: −14.5‰, $\delta^{15}N$: 12.3‰] and TR-05 from

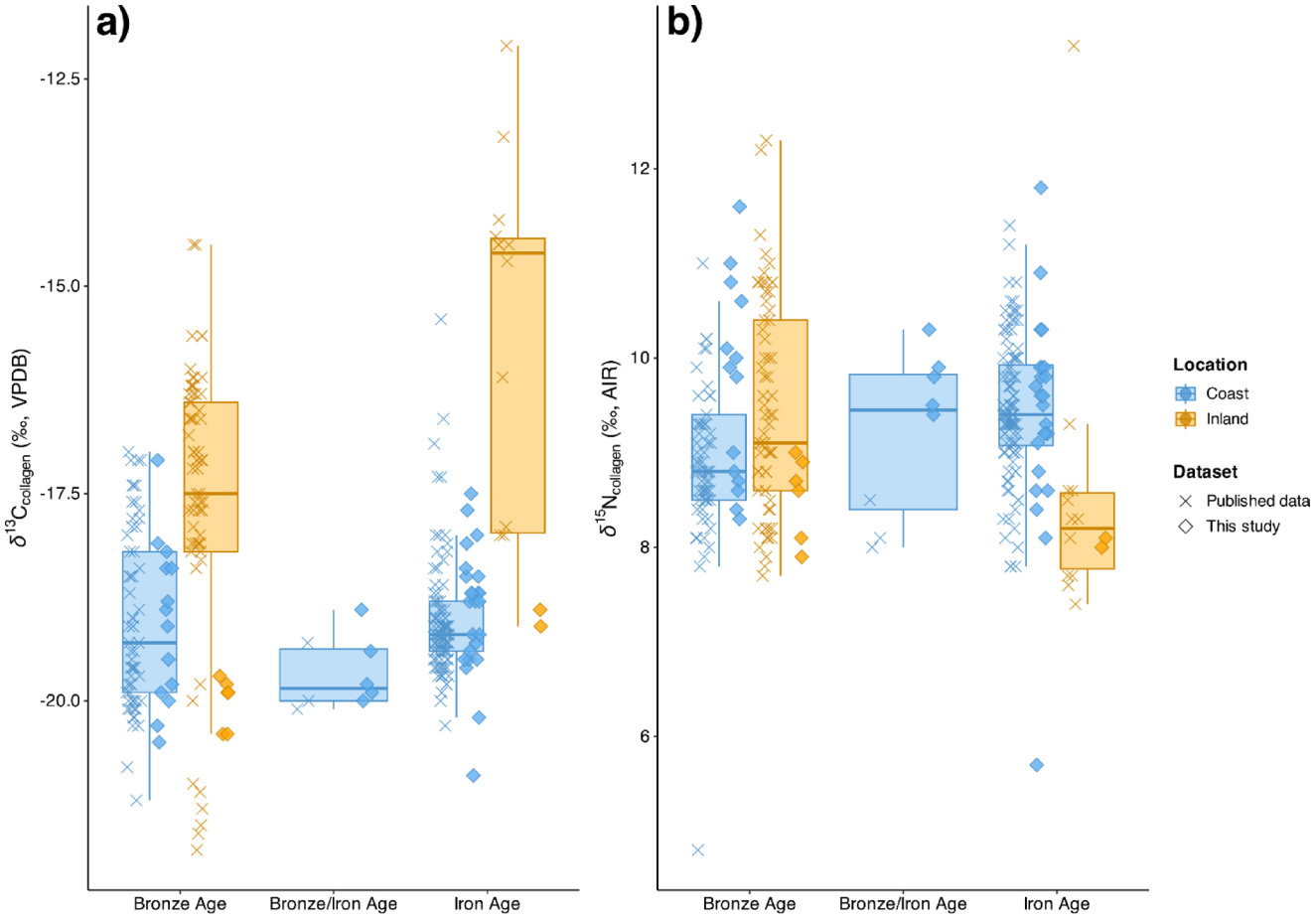

Fig. 4. Boxplots of (**a**) δ^{13}C and (**b**) δ^{15}N human values by period and location. Points represent individual samples, with shapes distinguishing data generated from this study and previously published data.

Trošmarija [EIA; δ^{13}C: − 14.5‰, δ^{15}N: 13.3‰]). This pattern is consistent with previous dietary evidence from the Adriatic and, more generally, the southeastern Mediterranean, which indicates a limited reliance of coastal communities on marine resources as early as the Neolithic[58,59]. Exceptions include some seasonally-occupied sites for the targeted exploitation of specific species like Vela Spilja[60] and the consumption of low-trophic marine foods (e.g., molluscs) from the Zadar lowlands[61] during the Neolithic. However, this interpretation should be considered tentative. In the absence of isotopic baselines for both freshwater and marine organisms for the Bronze and Iron Ages, the lack of a strong isotopic signal cannot definitively exclude the consumption of marine resources, particularly low-trophic marine foods (such as shellfish or other invertebrates), especially in coastal contexts.

The faunal baseline shows herbivores consistently aligned with a C_3-based ecosystem. This indicates that the human C_4 signal reflects direct millet consumption rather than indirect incorporation through animal protein. Pigs, however, display slightly more positive δ^{13}C values, suggesting limited or occasional access to C_4 resources[40]. Notably, dogs exhibit contrasting patterns across periods: in the Bronze Age, their isotopic values are similar to those of humans, implying shared food sources and possible access to millet-derived resources (likely in the form of food scraps). In the Iron Age, however, dogs shift toward values more similar to those of pigs, suggesting a change in feeding practices. This shift could indicate that millet became more exclusively associated with human diets, while dogs were increasingly provisioned with refuse or secondary products based mainly on C_3 resources, although the limited and uneven dataset precludes firm conclusions.

To further investigate dietary patterns across periods and regions, we grouped individuals by site location (coastal vs. inland) (Fig. 5). The faunal baseline includes all samples discussed above, grouped in two categories (herbivores and omnivores) based on their feeding ecology.

Confirming the results discussed above, herbivores display isotopic signatures that are consistently distinct from those of humans, showing no evidence for C_4 plant consumption at any point during either the Bronze or Iron Age. In contrast, omnivores overlap partially with humans in their δ^{13}C values, particularly at coastal sites, indicating possible limited access to C_4 resources. However, their δ^{15}N values remain consistently below the human average, suggesting lower trophic-level protein intake compared to humans.

Among humans, inland populations exhibit clear isotopic evidence for millet consumption, first becoming significant during the MBA/LBA transition and gradually declining until the LBA/EIA. It should be emphasized, however, that this pattern is primarily visible in the combined regional dataset, as the newly analyzed sample

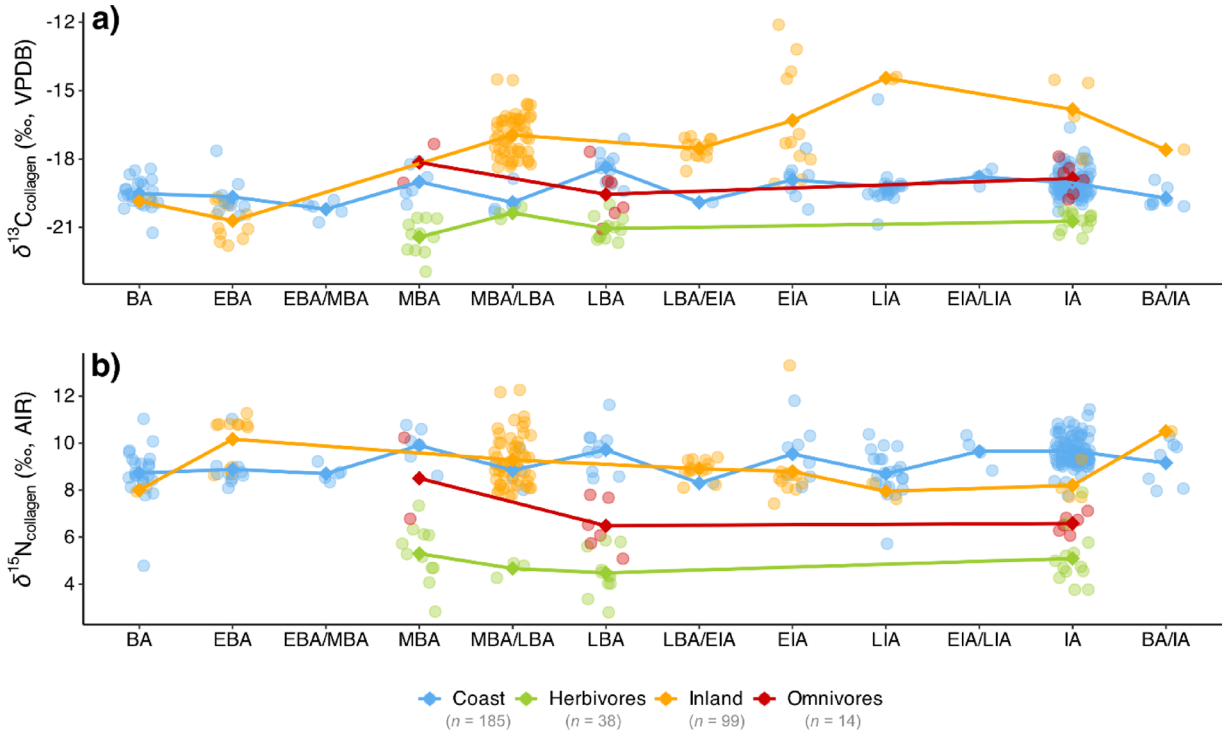

Fig. 5. (**a**) $\delta^{13}C$ and (**b**) $\delta^{15}N$ isotope results for the individuals from this and previously published papers[18,28,37,46,47,56], divided by location (coast vs. inland sites) and period. Baseline faunal data are divided into herbivores versus omnivores. Solid diamonds represent mean values per period and location.

alone does not show a clear $\delta^{13}C$ increase during the MBA/LBA transition, possibly due to the limited and uneven representation of key phases in the newly generated dataset.

A second increase appears during the EIA, with $\delta^{13}C$ values reaching their highest values in the LIA, although these results must be taken with caution as only two individuals represent the LIA in inland sites. By contrast, coastal populations show little or no reliance on millet throughout either the Bronze or Iron Ages. Although $\delta^{15}N$ values show a statistically significant difference between inland and coastal groups, this difference is modest and does not indicate fundamentally different protein sources across regions. These data suggest that dietary protein was primarily derived from terrestrial animals rather than aquatic resources, irrespective of geographical location.

To formally test whether dietary differences between inland and coastal populations were statistically significant across periods, two-way ANOVAs were conducted on $\delta^{13}C$ and $\delta^{15}N$ values, with Period and Location as factors. These analyses were complemented by non-parametric Kruskal–Wallis tests and pairwise Wilcoxon rank-sum tests to account for potential violations of normality.

Statistical analyses reveal pronounced structure in $\delta^{13}C$ values driven by both chronology and location. Two-way ANOVA shows significant effects of Period, Location, and their interaction (Supplementary Information S3), indicating that diachronic dietary trends differ between inland and coastal contexts. Non-parametric tests confirm substantial heterogeneity among Period/Location groups. Inland individuals generally exhibit more enriched $\delta^{13}C$ values relative to coastal groups, supporting greater incorporation of C_4 plants. However, this pattern should be interpreted with caution given the limited number of inland individuals in the dataset. These differences likely reflect a combination of baseline environmental variation and increased incorporation of C_4 resources. Temporal variation is also evident, with significant differences between several Period/Location groupings, although these are not uniformly distributed through time, indicating a heterogeneous and non-linear pattern of C_4 plant consumption.

For $\delta^{15}N$, statistical tests indicate a significant effect of Period and a Period/Location interaction, while Location alone is not significant. This suggests that nitrogen isotope variability is structured primarily through time, but that differences between inland and coastal populations vary depending on the chronological phase rather than reflecting a consistent spatial pattern. Although selected temporal differences are observed in both inland and coastal groups, these are less systematic than for $\delta^{13}C$ and generally modest in magnitude ($\leq \sim 1‰$). Overall $\delta^{15}N$ values are broadly consistent with predominantly terrestrial protein consumption, indicating that observed variability more likely reflects phase-specific differences in animal husbandry or local ecological baselines rather than sustained exploitation of aquatic resources.

How much did millet matter?

Given the absence of direct isotopic baselines for C_4 plants in prehistoric sites of the Eastern Adriatic, we applied a two-endmember mixing model to estimate millet contributions, following the theoretical framework outlined

by Laffranchi and colleagues[30] (Supplementary Information S3). The mixing model revealed consistently low millet intake across most periods (Supplementary Information S4), with two notable phases of increase (Fig. 6). Median C_4 contributions (with 50% and 95% credible intervals [CI]) indicate that C_4 plant intake was negligible in the EBA and MBA, remaining at or close to zero in the EBA (~0.3%, 95% CI 0.0–2.2%, n = 22), EBA/MBA (0.0%, 95% CI 0.0–0.6%, n = 5), and MBA (0.9%, 95% CI 0.0–9.1%, n = 6), suggesting diets were overwhelmingly based on C_3 resources.

A first pronounced shift occurs at the MBA/LBA transition, where median contributions rise to ~11% (95% CI 2.6–25.9%, n = 59). These values therefore likely reflect moderate incorporation of C_4-derived carbon into dietary protein (as these estimates derive from collagen $\delta^{13}C$ values, they reflect the protein component of the diet rather than total caloric intake) and should not be taken to indicate that millet constituted a dominant component of the overall diet. Nonetheless, they mark the first clear isotopic signal of millet as a non-negligible contribution of C_4-derived carbon to human diets, accompanied by substantial inter-individual variability. The subsequent LBA shows a return to lower values (~2.7%, 95% CI 0.0–14.7%, n = 10), although it must be noted that some individuals still fall within the elevated C_4 range and this phase is only represented by coastal individuals. The LBA/EIA transitional group maintains moderately high values (~7.1%, 95% CI 0.0–20.7%, n = 14), and the EIA reveals the second major increase in C_4 plant contributions, with median values again ~11% (95% CI 5.3–24.6%, n = 19). This suggests renewed or regionally intensified millet use during the EIA.

Later phases show a general decline, though with continued variability. The LIA presents moderate values (~3.9%, 95% CI 2.1–11.6%, n = 23), while the EIA/LIA transitional group drops to near-zero levels (~0.2%, 95% CI 0.0–10.9%, n = 4). The IA overall shows low contributions (~1.6%, 95% CI 0.5–10.0%, n = 89), indicating limited reliance on C_4 crops, albeit with wide confidence intervals pointing to heterogeneous consumption practices across sites and individuals.

These results suggest that millet was absent or negligible in the early phases of the Bronze Age, entered the diet episodically in the MBA/LBA and EIA, and in between and thereafter remained only a minor or occasional dietary component. This trajectory aligns with studies suggesting that the cultivation and consumption of broomcorn millet in Croatia during the Bronze Age were patchy and likely shaped by local environmental and cultural conditions[17,21]. The peaks observed at the MBA/LBA transition and during the EIA may reflect localized episodes of intensified millet use, perhaps tied to adaptive agricultural strategies, seasonal risk buffering, or specific social practices, rather than a sustained or widespread dietary transformation.

This interpretation is subject to several limitations. First, chronological resolution remains uneven across periods, with some phases (e.g., MBA/LBA and IA) represented by larger sample sizes than others. This imbalance affects the precision of estimates, influencing the width of credible intervals and the comparability of C_4 contributions between periods. Second, the mixing model relies on standardised isotopic values for C_4 plants in the absence of region-specific baselines, which may not fully capture local environmental or agricultural variability. Third, collagen $\delta^{13}C$ values primarily reflect the isotopic composition of dietary protein rather than total caloric intake. As such, the estimated C_4 contributions represent the protein component of the diet and may underestimate the overall dietary importance of millet, which likely contributed primarily to carbohydrate intake.

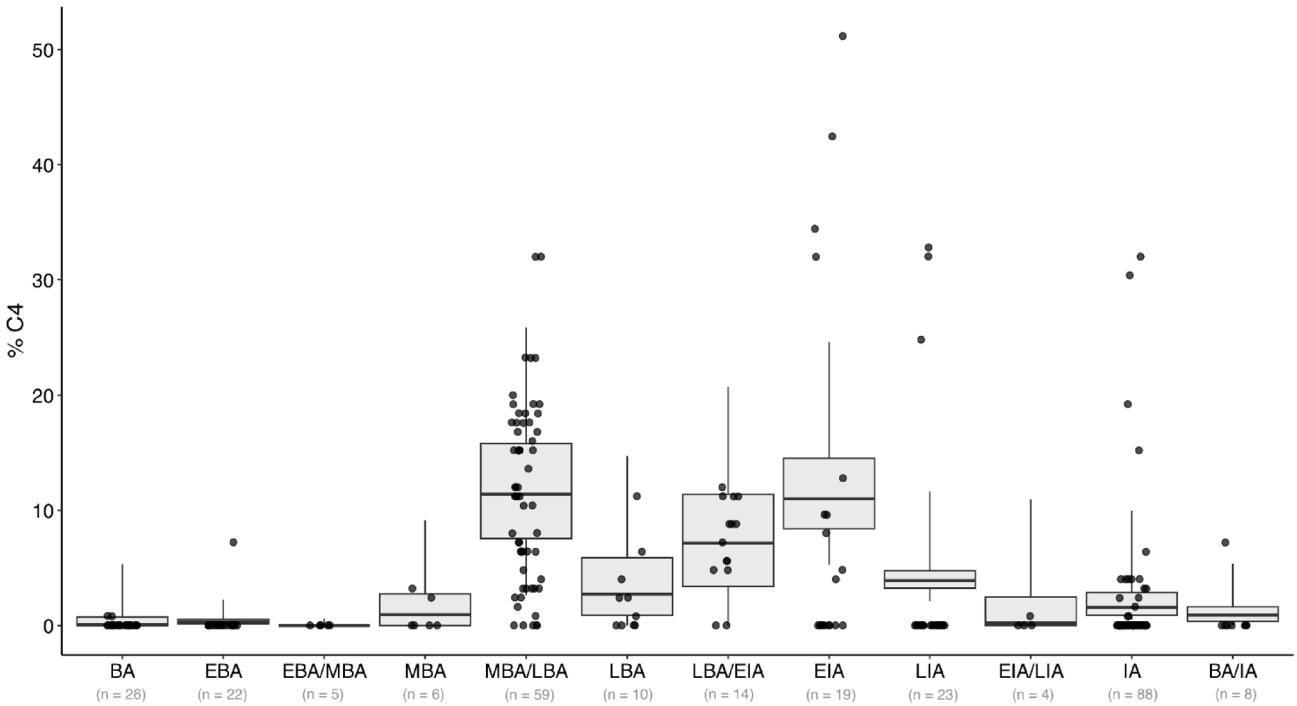

Fig. 6. Estimated percentage contribution of C_4 plants during the Bronze and Iron Ages, by period.

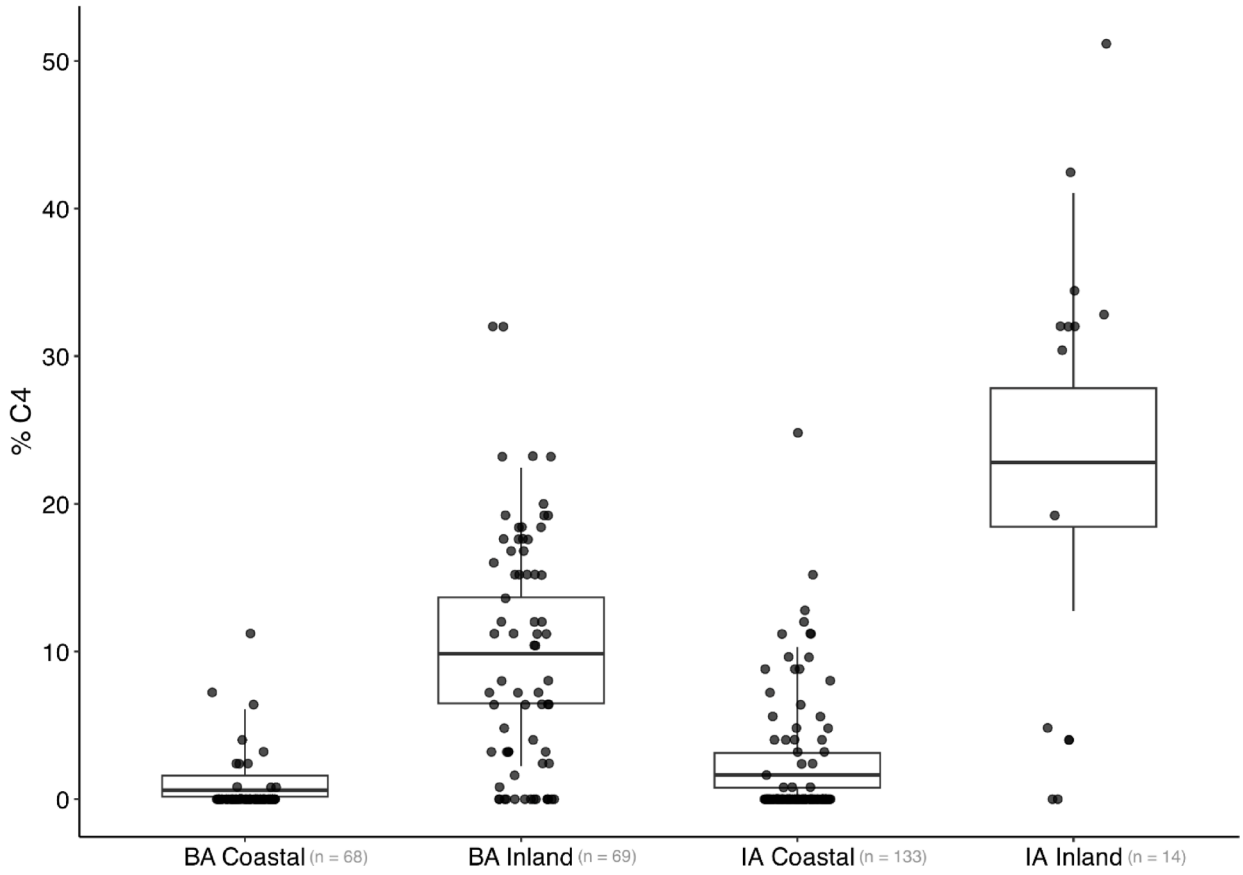

Fig. 7. Estimated percentage contribution of C_4 plants during the Bronze and Iron Ages, by location.

Despite these limitations, the results refine previous interpretations of millet's introduction in the region. Earlier studies[17–19,21] proposed a LBA arrival of millet in Croatia, with higher levels of consumption in inland areas during the Iron Age[18]. However, the present data point to the MBA/LBA transition as the earliest clear signal of millet consumption, in keeping with findings by Zavodny and colleagues[37] and thereby shifting the chronology of its adoption back by several centuries than previously thought on the basis of archaeobotanical remains alone[17,19]. This interpretation is also consistent with isotopic evidence from sites such as Bezdanjača Cave[46,62], where MBA/LBA individuals show clear C_4 inputs. At the same time, the more extensive dataset presented here challenges previous claims[37] of very high millet consumption throughout the Iron Age. When evaluated across a broader range of sites, the isotopic signal of millet consumption appears more modest, supporting a scenario in which millet remained a supplemental rather than a dominant component of subsistence economies.

The mixing model also highlights a marked contrast between coastal and inland communities in the extent of C_4 plant consumption throughout the Bronze and Iron Ages (Fig. 7; Supplementary Information S5). It should be noted, however, that for this comparison the Bronze Age is treated as a single analytical unit, which necessarily masks the diachronic variability in millet consumption observed between its earlier and later phases. As such, the results presented here provide a broad spatial comparison rather than a temporally resolved reconstruction. Bronze Age coastal groups show virtually no evidence of millet intake, with a mean contribution of less than 1% (95% CI 0–6.0%), consistent with a wholly C_3-based diet. By contrast, Bronze Age inland groups show higher estimated contributions, with a mean of 9.8% (95% CI 2.2–22.4%), suggesting a greater, though variable, incorporation of millet in continental settings. In the Iron Age, coastal groups again exhibit negligible input (~1.6%), indicating continuity in subsistence strategies based on C_3 plants, while inland groups show higher estimated contributions, on average, with a mean of almost 23% (95% CI ~12.7–41.1%). While these results point to a greater role for millet in inland areas, particularly during the Iron Age, they should be interpreted with caution given the wide credible intervals and limited sample size. Overall, millet appears to have remained marginal along the coast but may have played a more consistent, though still variable, role in inland dietary practices.

Although inland and coastal sites show differences in estimated C_4 plant consumption, regression analyses show no linear relationship between $\delta^{13}C$ values and distance from the coast (Supplementary Information S3). This indicates that millet adoption was not structured along a simple linear gradient from the coast, but instead followed a patchy, regionally differentiated pattern. This aligns with radiocarbon evidence[21] for the uneven spread of broomcorn millet along an east–west axis, reaching continental Croatia earlier than the Adriatic coast.

The broad 95% credible intervals observed for inland groups further highlight substantial heterogeneity, suggesting that millet was not ubiquitously cultivated or consumed but was instead an occasional or locally

significant crop whose role varied across sites and communities, as reflected in variable incorporation of C_4-derived carbon. While the limited number of inland individuals requires cautious interpretation, these patterns point to a more heterogeneous and context-dependent use of millet.

The comparatively lower and more sporadic signals observed in coastal areas may reflect both ecological and political factors. In the inland zones of the Eastern Adriatic (dominated by karst, slopes, and marginal soils)[61], intensified land use required flexible and resilient subsistence strategies. The episodic incorporation of millet in these inland settings, especially during the MBA/LBA transition and the Early Iron Age, may therefore be understood not as a primary driver of agricultural change but as a supplementary crop embedded within broader strategies of risk management and landscape investment as a response to fluctuating and agriculturally challenging weather conditions[12,13]. By contrast, coastal communities, increasingly integrated into wider Mediterranean exchange networks during the first millennium BCE[2,4,8], potentially buffered by low-trophic or seasonal marine resources, show little evidence for comparable dietary diversification.

Conclusions

This study provides the first comprehensive, regional-scale comparative stable isotope assessment of human and faunal remains from Bronze and Iron Age in the Eastern Adriatic. Here, we demonstrate that broomcorn millet was absent or negligible in Early Bronze Age diets and entered human subsistence during the Middle/Late Bronze Age transition. Additionally, while coastal communities show little to no evidence of millet consumption throughout the Bronze or Iron Ages, inland groups display consistently higher $\delta^{13}C$ values and a clear increase in C_4 input during both the MBA/LBA transition and the Iron Age, although these increases were neither continuous nor uniform. Faunal baselines confirm that this isotopic signal reflects direct human consumption rather than indirect incorporation via animal foddering.

Overall, our results refine the chronology of millet's adoption in the Eastern Adriatic, pushing its earliest significant dietary contribution back from the Late Bronze Age to the MBA/LBA transition. They also reveal that millet's role was context-dependent (i.e., significant in inland regions but marginal along the coast) and adopted intermittently to complement traditional C_3-based subsistence.

The diachronic patterns of millet consumption observed in human, but not faunal, isotopic values suggest that C_4 plant intake was a deliberate dietary choice rather than a passive response to agricultural availability. Several factors may account for this pattern. First, millet's short growing cycle, drought resilience, and nutritional profile would have made it a valuable risk-buffering resource during periods of climatic instability (particularly under the cool and wet conditions of the Sub-Boreal period), which may explain its episodic rather than sustained incorporation into human diets. Second, the restriction of elevated $\delta^{13}C$ values primarily to inland populations suggests that millet adoption was shaped by local ecological conditions and subsistence needs, with coastal communities having greater access to alternative dietary resources and thus less incentive to integrate C_4 crops. Third, the absence of a C_4 signal in fauna, especially in omnivores such as dogs and pigs that were directly provisioned by humans, suggests that millet was deliberately and exclusively allocated to human consumption, suggesting that it held particular value within these communities, whether nutritional, economic, or social.

To investigate this further, future research expanding isotopic coverage to other regions of Croatia could assess whether millet adoption followed similar trajectories elsewhere or if distinct regional patterns existed. Additionally, combining isotopic data with archaeobotanical evidence and palaeoclimatic reconstructions could test these hypotheses more rigorously, particularly by examining whether peaks in millet consumption correlate with periods of climatic stress or social reorganisation in the region. Lastly, integrating mobility studies (e.g., through strontium or sulfur isotope analyses) could help determine whether millet consumption was associated with specific population groups, whether locals or non-locals, shedding further light on the social dimensions of C_4 plant use in prehistoric Croatia.

Methods

Stable isotope analysis

Stable isotope analysis has long become one of the most widely applied methodologies in archaeology for reconstructing ancient diets and mobility patterns. Its interpretive strength derives from predictable isotopic fractionation (i.e., the preferential incorporation or exclusion of lighter versus heavier isotopes during biochemical processes)[63]. Fractionation produces systematic differences in isotopic composition among primary producers, consumers, and their tissues, allowing dietary inferences from human and faunal remains. Analytical results are expressed in delta (δ) notation, which quantifies the deviation in the ratio of heavy to light isotopes ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) in a sample relative to internationally recognized standards. $\delta^{13}C$ values are reported relative to the Vienna Pee Dee Belemnite (VPDB) standard, while $\delta^{15}N$ values relative to atmospheric nitrogen (AIR), with values expressed in per mil (‰).

Carbon isotope ratios ($\delta^{13}C$) are especially informative because photosynthetic pathways fractionate carbon isotopes to different extents. C_3 plants, which include most temperate cereals such as wheat and barley, fix carbon through the Calvin–Benson cycle using the enzyme RuBisCO[64], which discriminates strongly against ^{13}C. This results in relatively low $\delta^{13}C$ values (*c.* −26 to −28‰ under pre-industrial atmospheric conditions)[30,65–67]. In contrast, C_4 plants such as millets utilize the Hatch–Slack pathway, in which carbon fixation is mediated by PEP carboxylase[64], an enzyme that exhibits far less discrimination against ^{13}C. Consequently, C_4 plants yield much higher $\delta^{13}C$ values (*c.* −11 to −14‰)[30,58,65–67].

Interpretation of $\delta^{13}C$ values in collagen, however, requires consideration of the trophic discrimination factor (TDF), which represents the isotopic offset between diet and consumer tissues ($\Delta^{13}C_{tissue\text{-}diet}$). For carbon, collagen ^{13}C values are typically enriched relative to diet, but controlled feeding experiments and modern dietary studies indicate that the magnitude of this offset is variable[58]. Early work suggested an enrichment of

approximately + 1–3‰[68], but subsequent studies have demonstrated that $\Delta^{13}C_{tissue\text{-}diet}$ can range from + 0.5‰ to as much as + 7‰[69,70], depending on dietary macronutrient composition.

Nitrogen isotope ratios ($\delta^{15}N$) provide complementary dietary information. Fractionation during amino acid deamination and nitrogen excretion results in predictable enrichment of ^{15}N with each trophic transfer. Unlike carbon, nitrogen fractionation is largely independent of photosynthetic pathway, but $\delta^{15}N$ values are influenced by ecological and cultural variables, including aridity[71], soil management (e.g., manuring)[72,73], and animal husbandry practices[74,75]. Offsets between diet and collagen are generally less variable than for carbon, averaging + 3.4 ± 1.0‰[68,76,77], though higher enrichments (up to + 6‰) have been reported in controlled human dietary studies[78].

Samples for $\delta^{13}C$ and $\delta^{15}N$ analysis were prepared at the Penn State University Human Palaeoecology, Isotope Geochemistry Laboratory and at the Paleoecology and AMS Radiocarbon Research Facility, University of California, Santa Barbara, and the Center for Applied Bioarchaeology at the Institute for Anthropological Research in Zagreb, Croatia. Long bones were preferentially selected when available; otherwise, fragments of ribs, skull, or short bones (metatarsals) were used. At least 0.5 g of dry cortical bone was taken from each specimen and mechanically cleaned to remove surface contaminants. The samples were then crushed to increase the reactive surface area, rinsed with purified water, and demineralized in 0.5 M HCl at 5 °C until complete demineralization. Collagen was extracted following a modified Longin[79] protocol, with the addition of an ultrafiltration step[80].

Poorly preserved bone collagen samples (JAZ1A, JAZDA, KUKU4, STT2G1A, STT2G1B, STT3, STT4, OGFAU2) that could not undergo ultrafiltration were processed using a modified XAD-purification method[81–83] In this procedure, gelatin was hydrolyzed in 1.5 mL 6 M HCl for 24 h at 110 °C, passed through a solid-phase extraction (SPE) column with an additional 10 mL 6 M HCl, and dried under UHP N_2 gas.

Carbon and nitrogen concentrations and stable isotope ratios were measured at the University of New Mexico Center for Stable Isotopes (UNM-CSI, USA), at the Yale Analitical and Stable Isotope Center (YASIC), and at Vilnius Radiocarbon (Lithuania). At YASIC, measurements were made using a Thermo DeltaPlus Advantage continuous flow isotope ratio mass spectrometer coupled to a Costech ECS 4010 Elemental Analyser with CONFLO III interface. At CSI-UNM measurements were made using a ThermoFisher Scientific Delta V Advantage mass spectrometer coupled to a Costech ECS 4010 Elemental Analyzer with CONFLO IV interface. In both cases, stable carbon and nitrogen isotopic compositions were calibrated relative to the Vienna Pee Dee Belemnite (VPDB) and American Institutes for Research (AIR) scales using USGS40 and USGS41. $\delta^{13}C$ and $\delta^{15}N$ values are reported in standard ‰ notation with respect to VPDB and atmospheric nitrogen, respectively.

At YASIC measurement uncertainty was monitored using YASIC reference material collagen standards with characterized isotopic compositions: Carbon Nitrogen reference 2 (glutamic acid isotope reference; $\delta^{13}C$: − 28.3‰, $\delta^{15}N$: − 4.5‰), Yale Glutamic Acid (glutamic acid isotope reference; $\delta^{13}C$: − 31.0‰, $\delta^{15}N$: 31.8‰), Trout (muscle tissue; $\delta^{13}C$: − 29.1‰, $\delta^{15}N$: 15.7‰). Precision was determined to be ± 0.18 for $\delta^{13}C$ and ± 0.08 for $\delta^{15}N$ based on repeated measurements of check standards. Accuracy was determined to be ± 0.13 for $\delta^{13}C$ and ± 0.11 for $\delta^{15}N$ on the basis of the difference between the observed and known δ values of the check standards and their long-term standard deviations. The total analytical uncertainty was estimated to be ± 0.22 for $\delta^{13}C$ and 0.14 for $\delta^{15}N$ according to published equations[84,85].

At CSI-UNM the three internal laboratory standards were UNM-CSI Protein std#1 (casein purchased from Sigma Aldrich; $\delta^{13}C$: − 26.52‰; $\delta^{15}N$: 6.43‰), UNM-CSI Protein std#2 (soy protein purchased from Sigma Aldrich; $\delta^{13}C$: − 25.78‰; $\delta^{15}N$: 0.98‰), UNM-CSI protein Std#4 (house made tuna protein; $\delta^{13}C$: − 16.17‰; $\delta^{15}N$: 13.32‰). Here analyses were normalized to the laboratory standards which were calibrated against IAEA N1, IAEA N2 and USGS 43 for $\delta^{15}N$ and NBS 21, NBS 22 and USGS 24 for $\delta^{13}C$[86].

At Vilnius, samples were analyzed using an Elementar BioVisION system consisting of an isotope ratio mass spectrometer (Isoprime visION) coupled to an elemental analyzer (Vario Isotope Cube), configured for simultaneous carbon and nitrogen analysis. Reference materials USGS24 ($\delta^{13}C = -16.05 \pm 0.04$‰), IAEA-600 ($\delta^{13}C = -27.77 \pm 0.04$‰, $\delta^{15}N = 1.02 \pm 0.05$‰), and IAEA-N-2 ($\delta^{15}N = 20.41 \pm 0.12$‰) were used for $\delta^{13}C$ and $\delta^{15}N$ normalization to the VPDB (Vienna Pee Dee Belemnite) and Air-N_2 (atmospheric nitrogen) delta scales, respectively. Protein (casein) powder served as an in-house standard and was calibrated against the international reference materials IAEA-CH-6 and IAEA-N-1. The typical analytical precision was better than 0.15‰ (1σ) for $\delta^{13}C$ and 0.20‰ (1σ) for $\delta^{15}N$.

Isotope values are reported in δ-notation (‰) relative to internationally defined standards: Vienna Pee Dee Belemnite (VPDB) for carbon and Ambient Inhalable Reservoir (AIR) for nitrogen. Only samples with an atomic C:N ratio between 2.9 and 3.6, concentrations of C (%C) and N (%N) greater than 13% and 4.8%, and collagen yields greater than 2%[76,87–89] were considered suitable for analysis (Supplementary Information S2). Three faunal samples had %N values below the recommended threshold; however, they were retained as they exhibited acceptable C:N ratios and %C values, and showed no other indications of poor collagen preservation. In addition, their isotopic values are consistent with those expected for herbivorous species, supporting the reliability of these measurements. Collagen quality was therefore assessed holistically, following established recommendations[90] that multiple indicators should be considered when evaluating sample preservation.

Chronology and radiocarbon dates

The chronology of sites and individuals is based on a combination of archaeological context and direct radiocarbon analysis. Radiocarbon dates were obtained by AMS at the Keck Carbon Cycle AMS Facility (UCIAMS), Penn State University (PSUAMS), Beta Analytic (Beta), and Vilnius Radiocarbon (FTMC). Conventional ages (BP) were calibrated using the IntCal20 calibration curve[91]. The resulting calibrated ranges (68.2% and 95.4% probability) were then assigned to archaeological periods (Early, Middle, and Late Bronze Age; Early and Late Iron Age) following regional chronologies. In cases where radiocarbon dates were not available and chronology

was inferred from archaeological context, samples are referred to more general categories (e.g., "Bronze/Iron Age" or "Iron Age"). A complete list of radiocarbon results is provided in Supplementary Information S2.

Maps, plots and statistical analyses

The map showing the location of the sites considered in this study was produced using QGIS (version 3.24.2-Tisler), using the Esri ocean baseline. Site coordinates were retrieved from EPSG.io (https://epsg.io/) using WGS 84/ UTM zone 34N EPSG:32634. Isotope plots, and statistical analyses were generated in RStudio (version 4.3.1).

To evaluate potential differences in stable isotope values between groups (e.g., adults vs. subadults, inland vs. coastal, and across chronological periods), we applied parametric tests (Student's t-test for two groups; one-way ANOVA with Tukey's HSD post hoc tests for more than two groups) (Supplementary Information S3). Parametric approaches were selected because isotopic data are typically approximately normally distributed and because we aimed to test differences in means, which are most directly interpretable in dietary studies.

Then, to evaluate differences in stable isotope values across time and space for these and previously published isotope results, we performed statistical analyses on human collagen $\delta^{13}C$ and $\delta^{15}N$ values. Periods were coded according to archaeological chronology (Early Bronze Age to Iron Age), and individuals were assigned to either inland or coastal groups based on site location. Normality and homogeneity of variance were first assessed through visual inspection of residuals and Levene's test. Because assumptions were not fully met, we used a combined approach. First, a two-way ANOVA was applied to test for the main effects of "Period" and "Location" (inland vs. coast), as well as their interaction, on $\delta^{13}C$ and $\delta^{15}N$ values. Then, to account for non-normal distributions and unequal group sizes, we also ran non-parametric tests. A Kruskal–Wallis test was conducted on $\delta^{13}C$ and $\delta^{15}N$ values across all period-location combinations, followed by pairwise Wilcoxon rank-sum tests with Benjamini-Hochberg (BH) correction for multiple comparisons.

Mixing model

As no isotope values for C_4 plants are available for the Bronze and Iron Ages in the Eastern Adriatic, to assess the potential contribution of C_4-derived resources to human diets we applied a probabilistic two-endmember mixing model using collagen $\delta^{13}C$ values from our assemblage (code available in Supplementary Information S3). A local herbivore collagen mean value calculated taking into account terrestrial animals from the present and previously published papers from BA and IA Croatia (mean $\delta^{13}C = -21.0‰$, SD = 0.6‰, n = 38) was used as the empirical anchor for the C_3 baseline. Because human collagen is typically enriched relative to local herbivore collagen through routing and trophic processes, we applied an offset of +2.5‰ (±0.5‰) following Laffranchi and colleagues[30]. The C_4 endmember was modelled as $C_3 + \Delta C_4$, with ΔC_4 drawn from the literature[92] (mean = 12.5‰, SD = 2.0‰). For each human individual, we generated 100,000 Monte Carlo draws of C_3, C_4, and the observed $\delta^{13}C$ value (incorporating ±0.2‰ measurement error) and calculated the proportion of C_4-derived input in collagen (P_{C4}) as:

$$P_{C4} = \frac{d^{13}C_{sample} - d^{13}C_{C3}}{d^{13}C_{c4} - d^{13}C_{C3}}$$

with values constrained between 0 and 1. For each posterior distribution, we report the median, interquartile range (50% CI), and 95% credible interval. Summaries were calculated at both the individual level (Site) and aggregated by location (coast vs. inland) and by period (Supplementary Information S4 and Supplementary Information S5). To classify individuals with elevated $\delta^{13}C$ values, we applied two threshold criteria. In European contexts, human bone collagen values of ~20.5‰ are typical of fully terrestrial C_3-based diets, while predominantly C_4-based diets are expected to produce values close to −12‰[92] after accounting for trophic enrichment. We therefore used $\delta^{13}C \geq -12‰$ as a conservative cutoff for identifying individuals with predominantly C_4 diets. However, given natural variability in C_4 plant signatures and uncertainty in diet-collagen offsets (±2‰), collagen $\delta^{13}C$ values ≥ −14‰ can also plausibly reflect a high contribution of C_4 resources. For transparency, we report results using both thresholds: −12‰ as the strict criterion and −14‰ as a more inclusive estimate that incorporates expected isotopic variation.

Lastly, to visualize the posterior estimates of C_4 contributions, we plotted boxplots summarizing the two-endmember model outputs using *ggplot2*. For each group (Location, Period), boxplots show the posterior median (line), interquartile range (box), and 95% credible interval (whiskers), with individual medians overlaid as points.

Data availability

All data generated or analysed during this study are included in this published article (and its Supplementary Information files).

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Author contributions

Conceptualization: M.N., V.M.; Data curation: V.M., E.Z., S.M.; Formal analysis: S.M., M.C.; Funding acquisition: M.N.; Investigation: V.M.; Methodology: V.M., M.N., S.M.; Project administration: M.N.; Resources: M.Č., N.Č., S.G., D.P., D.T.P., M.V., J.Z., U.Z.A., B.Č., M.U.D.; Software: V.M.; Validation: V.M., M.N., E.Z., S.M.; Visualization: V.M.; Writing—original draft: V.M., M.N.; Writing—review and editing: E.Z., S.M., M.C., M.Č., N.Č., S.G., D.P., D.T.P., M.V., J.Z., U.Z.A.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to V.M.

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