Stable carbon and nitrogen isotopes identify nuanced dietary changes from the Bronze and Iron Ages on the Great Hungarian Plain

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The Great Hungarian Plain (GHP) served as a geographic funnel for population mobility throughout prehistory. Genomic and isotopic research demonstrates non-linear genetic turnover and technological shifts between the Copper and Iron Ages of the GHP, which influenced the dietary strategies of numerous cultures that intermixed and overlapped through time. Given the complexities of these prehistoric cultural and demographic processes, this study aims to identify and elucidate diachronic and culture-specific dietary signatures. We report on stable carbon and nitrogen isotope ratios from 74 individuals from nineteen sites in the GHP dating to a ~ 3000-year time span between the Early Bronze and Early Iron Ages. The samples broadly indicate a terrestrial C3 diet with nuanced differences amongst populations and through time, suggesting exogenous influences that manifested in subsistence strategies. Slightly elevated δ15N values for Bronze Age samples imply higher reliance on protein than in the Iron Age. Interestingly, the Füzesabony have carbon values typical of C4 vegetation indicating millet consumption, or that of a grain with comparable δ13C ratios, which corroborates evidence from outside the GHP for its early cultivation during the Middle Bronze Age. Finally, our results also suggest locally diverse subsistence economies for GHP Scythians.

The Great Hungarian Plain (GHP) forms a lowland confluence connecting the Balkans, Pontic Steppe, and Central Europe12. The GHP acted as a geographic funnel for population movement whereby new people and their ideas and ways of life, including subsistence...
strategies, migrated through Europe. As such, it functioned as a region of major cultural and technological transition throughout prehistory. It is thus a crucial region for identifying and investigating dietary trends between prehistoric time periods and cultures. We report on carbon and nitrogen stable isotope values from 74 individuals from nineteen sites in the GHP (Fig. 1) dating to a ~3000-year transect between the Early Bronze and Early Iron Ages (Table 1). Specifically, we address two main questions: (1) Can nuanced dietary changes be detected across millennia? (2) If changes are detected, what do they imply regarding prehistoric trans-Carpathian cultural communication and trade?

Stable Isotopes. The application of stable isotopes to prehistoric dietary analyses is complex as many factors affect the ratios obtained from samples. In brief, organisms incorporate carbon and nitrogen from diet material. For carbon, most plants fall under one of two categories (C3 and C4) based on their photosynthetic pathway, which makes it possible to distinguish general plant groups. C3 plants, which include temperate grasses and domesticated cereals from terrestrial ecosystems, exhibit carbon isotope values (13C/12C ratio compared to the standard VPDB or δ13C) from −38 parts per mil (‰) to −22‰, with a mean of −26.5‰. C4 plants, such as maize, sorghum, and millet, exhibit higher δ13C ratios, ranging between −21 and −9‰, with a mean of −12.5‰. Dietary nitrogen (15N/14N ratio compared to the standard AIR or δ15N) is incorporated via protein at a stepwise factor of about +3 to 5‰. Plants in some terrestrial ecosystems can range between −15 and −10‰, however, aquatic resources—including freshwater—can exhibit comparatively 15N-enriched values due to the relative complexity of the foodweb. Moreover, both nitrogen and carbon isotope ratios are affected by climate, soil conditions, elevation, water stress, health of the individual, and breastfeeding. Lastly, milk consumption, not unlike breastfeeding, augments δ15N values much like that seen in other dietary trophic level increases.
Cultural and dietary context. Animal husbandry, primarily of cattle, was the predominant subsistence practice during the Middle Copper Age of people migrating to the Carpathian Basin from the Eastern Steppe. The contemporaneous cultures of the Late Copper Age, including the Baden, Vučedol, and Cojofeni, continued the tradition of animal husbandry and land cultivation. A transformation from the ‘monolithic’ Baden culture to more varied and smaller regional Bronze Age communities was shaped either by internal developments or foreign influences, including population movement, of, for example, the Ynamaya, who arrived from the east during the Transitional Period (~ 2800 to 2600 BCE). This was followed by the expansion of the Bell Beaker who migrated from the west (~ 2500 BCE), and was accompanied by several independent cultures (e.g., Makó-Kosihy-Caka, Nagyrév, Somogyvár-Vinkovici), all practicing intensive cereal cultivation and animal husbandry. However, only small groups settled along Danube River routes.

The transition from the Early Bronze to Middle Bronze Age is marked by the development of the more sedentary Hatvan, Otomani/Ottomány, and Füzesabony cultures, all of whom occupied tells. These groups and others coexisted for centuries, although not necessarily peacefully, until the end of the Middle Bronze Age. The Füzesabony were partly contemporaneous with and subsequent to the Hatvan, with no indications that upon their arrival they usurped the former culture. The Otomani-Füzesabony is associated with increased socio-political and metallurgical complexity in the Carpathian Basin, as evidenced by tell sites, communal cemeteries, and advanced trade networks. By this point the plow had been introduced, with communities cultivating cereals like wheat and barley, vegetables and fruits, and likely fodder crops to feed cattle, pig, goat, sheep, and horses.

Although there was profound cultural diversification during the Early Bronze and Middle Bronze Ages, by the Late Bronze Age cultures appear to homogenize over large geographic regions, much like that which occurred between the Late Neolithic and Early Copper Age, as manifested in the reduction of local cultural expression. The emergence of several cultures, including the Piliny/Kyjatice in the northern mountain range, and Gáva east of the Tisza, likely resulted from interregional contacts between groups occupying different ecological zones, resulting in increased trade and information flow. This is further supported by the spread and increased cultivation of millet.

Late Bronze Age villages were seemingly abandoned, and new traditions and material culture appeared in the eastern parts of the Carpathian Basin at the beginning of the Iron Age (~ 900/800 BCE), namely on the central and southern part of the GHP, in the Northern Mountain Range, and in Transylvania. The Early Iron Age of the GHP is largely underrepresented in the archaeological record, perhaps because the cultures of this period, in particular the pre-Scythian (Mezőcsát), who mainly occupied the central and northern parts of the GHP, were nomadic stockbreeders of gregarious animals (e.g., cattle, sheep, horse), unlike their more sedentary predecessors. The Scythian (Vekerzug in the GHP) culture subsequently emerged and continued into the Middle Iron Age. Excavations of Vekerzug settlements indicate that agriculture and animal husbandry were practised along with highly developed iron metallurgy and ceramic manufacture. Various other Middle Iron Age cultures occupied this region until the end of the fifth century BCE, when the Celts began their conquests and interrupted development of local cultures, not just in the Tisza region, but throughout the Carpathian Basin. The associated cultures in the present dataset, and their associated dietary information, can be found in Table 1.

Previous archaeochemistry of the Great Hungarian Plain. To assess links between diet and cultural evolution on the GHP, stable isotope and ancient DNA (aDNA) research has been conducted on samples from the Neolithic through Iron Age. Previous carbon and nitrogen stable isotope analyses of human and faunal osteological samples from this region have focused primarily on Neolithic and Copper Age populations, reporting a transformation in subsistence strategies during the Late Neolithic and Copper Age towards increased consumption of animal protein compared to the previous subperiods. Gamba et al. analysed the genomes of thirteen GHP individuals dating to between the Early Neolithic and Early Iron Age; the Bronze and Iron Age samples provided evidence for genomic turnover that contrasted the genetic continuity observed during the Neolithic and Copper Age. Allentoft et al.’s study of Eurasian genomes reported dynamic migrations during the Bronze Age, as well as the rise of the allele that confers the lactase gene, while de Barros Damgaard et al. found that Scythian groups were genetically comprised of Late Bronze Age herders, farmers, and hunter-gatherers.

Comparing carbon and nitrogen isotopic ratios with aDNA results from GHP samples, Gamarra et al. found no associations between dietary, cultural, and genetic shifts from the Early Neolithic to Iron Age; however, Bronze and Iron Age individuals exhibited a diet higher in C4 plants, such as millet, which is a typical agricultural crop at this time in this region, compared to those from the Neolithic and Copper Age.

Genetic turnover, and technological evolution (e.g., changes in metallurgy during the Bronze Age: copper smelting to make Bronze, new casting techniques, sheet metal manufacture; and in the Iron Age: the ability to more locally produce iron, which affected political economies and the production of tools) were thus non-linear, influencing the dietary strategies of numerous cultures that intermixed and overlapped through time. Owing to the complexities of these prehistoric cultural and demographic processes, the present study thus aims to improve our understanding of diachronic and culture-specific dietary signatures as revealed by the archaeology and stable isotopes both between and within chronological periods and cultures.

Results
Archaeological and burial information are found in Supplementary Material S1, isotopic ratios, palaeodemographic information, and quality criteria are provided for each sample in Supplementary Material S2, and statistical tables are listed in Supplementary Material S3. Figure 2 illustrates the range of the stable carbon and nitrogen isotopic results of samples from this study coupled with data of Gamarra et al. and McCay. The range of δ13C ratios for the entire dataset is −21.2 to −14.8‰ (mean = −18.3‰ ± 1.6‰ (1σ)); the δ15N value range for the entire
dataset is +8.3 to 12.9‰ (mean = +10.5‰ ± 0.9‰ (1σ); Table 2). Overall, most samples indicate a terrestrial C₃ diet with nuanced statistical differences between certain groups, suggesting external influences that manifested in the diet. This is in keeping with what is known about food practices at the time, and is also congruent with previous isotopic analyses.34,58.

Figure 2. Scatterplots of human δ¹³C and δ¹⁵N ratios with mean δ¹³C and δ¹⁵N ratios (± 1 σ) by period (A) Bronze Age/BA (n = 50) and Iron Age/IA (n = 24) and subperiod (B) Early Bronze Age/EBA (n = 8), Middle Bronze Age/MBA (n = 18), Late Bronze Age/LBA (n = 22), and Early Iron Age/EIA (n = 24).

| Period/subperiod         | n  | δ¹³C range (‰) | Mean | SD  | δ¹⁵N range (‰) | Mean | SD  |
|--------------------------|----|----------------|------|-----|----------------|------|-----|
| Bronze Age               | 50 | −21.2 to −14.8 | −18.7| 1.5 | 8.9 to 12.9    | 10.7 | 0.9 |
| Iron Age                 | 24 | −20.5 to −14.9 | −17.5| 1.2 | 8.3 to 12.4    | 10.0 | 0.9 |
| Early Bronze Age         | 8  | −20.3 to −19.7 | −20.0| 0.2 | 9.3 to 12.9    | 10.7 | 1.2 |
| Middle Bronze Age        | 18 | −21.1 to −16.7 | −19.4| 1.3 | 8.9 to 12.2    | 10.7 | 0.9 |
| Late Bronze Age          | 22 | −19.9 to −14.8 | −17.7| 1.3 | 9.8 to 12.9    | 10.9 | 0.8 |
| Early Iron Age           | 24 | −20.5 to −14.8 | −17.5| 1.2 | 8.3 to 12.4    | 10.0 | 0.9 |
Chronological variability. The δ¹³C and δ¹⁵N results per period and subperiod are summarized in Table 2; tests of normality are found in Supplementary Material S3a, S3b, S3c, and S3d. There is an overall statistical difference for δ¹³C values between the Bronze and Iron Ages (Mann–Whitney U: U = 900.5; p = 0.001; Fig. 3A; Supplementary Material S3e); the Early Bronze, Middle Bronze, Late Bronze, and Early Iron Ages also exhibit significant statistical differences (Kruskal–Wallis:  X² = 31.122, p < 0.001; Fig. 4A; Supplementary Material S3f). As the Kruskal–Wallis test (p < 0.001) indicated differences between the four subperiods analyzed, pairwise Mann–Whitney tests were employed, revealing a significant difference between the Early Bronze and Middle Bronze Ages compared with the Late Bronze and Early Iron Ages (Supplementary Material S3g).

Similarly, for δ¹⁵N ratios there is an overall statistical difference between the Bronze and Iron Ages (independent samples t-test  t₁₃ = 3.369, p = 0.001; Fig. 3B; Supplementary Material S3h). The Tukey’s post hoc analysis (Supplementary Material S3i), performed after a significant ANOVA (F(3,68) = 4.250, p = 0.008) result (Supplementary Material S3j), identified differences between the Late Bronze and Early Iron Ages, and differences among the Early, Middle, and Late Bronze Ages (Fig. 4B). Furthermore, there is a negative correlation (− 0.418, p < 0.001) across the four subperiods when comparing mean δ¹³C and δ¹⁵N ratios, seen when the increase in overall δ¹³C values correlates with a decrease in δ¹⁵N values.

Cultural variability. The ranges for δ¹³C and δ¹⁵N ratios by culture are listed in Table 3; the δ¹³C and δ¹⁵N results are represented in Fig. 5; the tests of normality can be found in Supplementary Material S3k and S3l. For the inter-culture comparison (n = 67) there is an overall statistical difference between δ¹³C values (Kruskal–Wallis:  X² = 25.159, p < 0.001) with the Füzesabony found to be significantly different from the Gáva and Scythian (Fig. 5A; Supplementary Material S3m). Similarly, the Proto–Nagyérő compared with the Gáva, Pilíny/Kyjatice, pre-Scythian, and Scythian also demonstrate a notable difference in δ¹³C values (Pairwise Mann–Whitney, Supplementary Material S3n). The comparison of δ¹⁵N ratios revealed an overall statistical difference between cul-
Figure 4. Violin plot of human (A) $\delta^{13}$C and (B) $\delta^{15}$N ratios by subperiod: Early Bronze Age (n = 8), Middle Bronze Age (n = 18), Late Bronze Age (n = 22), and Early Iron Age (n = 24). Center black dot represents mean; center black line represents distribution.

| Cultures            | Time period(s)          | n  | $\delta^{13}$C range (‰) | Mean | SD   | $\delta^{15}$N range (‰) | Mean | SD   |
|---------------------|-------------------------|----|---------------------------|------|------|---------------------------|------|------|
| Nyírség             | Early Bronze Age        | 1  | $-20.2$                   | N/A  | N/A  | 9.3                       | N/A  | N/A  |
| Proto-Nagyrév       | Early Bronze Age        | 4  | $-19.8$ to $-19.7$        | $-19.8$ | 0.1 | 10.6 to 12.9             | 11.7 | 0.9  |
| Bell Beaker         | Early Bronze Age        | 2  | $-20.3$ to $-20.2$        | $-20.2$ | N/A | 9.3 to 9.8                | 9.5  | N/A  |
| Hatvan              | Early Bronze Age        | 1  | $-19.9$                   | N/A  | N/A  | 10.7                      | N/A  | N/A  |
| Hatvan or Füzesabony| Early Bronze Age/Middle Bronze Age | 6  | $-21.0$ to $-17.2$        | $-18.6$ | 1.6 | 9.4 to 11.3              | 10.0 | 0.7  |
| Füzesabony          | Middle Bronze Age       | 12 | $-21.1$ to $-16.7$        | $-19.4$ | 1.3 | 8.9 to 12.2              | 10.7 | 0.9  |
| Otomány/Ottomány    | Middle Bronze Age       | 1  | $-20.1$                   | N/A  | N/A  | 9.2                       | N/A  | N/A  |
| Piliny or Piliny/Kyjatice | Late Bronze Age      | 14 | $-19.8$ to $-16.1$        | $-18.0$ | 1.0 | 10.1 to 12.8             | 11.1 | 0.6  |
| pre-Gáva or Gáva    | Late Bronze Age, Early Iron Age | 8  | $-18.0$ to $-14.8$        | $-16.7$ | 1.2 | 9.8 to 12.9              | 10.7 | 1.0  |
| Pre-Scythian (Mezőcsát) | Early Iron Age        | 4  | $-18.1$ to $-14.8$        | $-16.8$ | 1.3 | 10.4 to 11.0             | 10.8 | 0.2  |
| Scythian (Vekerzug) | Early Iron Age          | 19 | $-20.5$ to $-15.6$        | $-17.7$ | 1.1 | 8.3 to 12.4              | 9.8  | 0.9  |

Table 3. Summary of human $\delta^{13}$C and $\delta^{15}$N results by culture with‰ range, mean, and SD (± 1σ).

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Demographic variability. Only adults of known sex were statistically assessed as too few individuals were identified for other demographic groups to be compared (i.e., age groups; Descriptive Information, Supplementary Material S3q; tests of normality, Supplementary Material S3r, S3s, S3t, and S3u). Between periods both females (n = 27) and males (n = 29) exhibit statistical differences for δ13C values (Mann–Whitney U: U = 42.5, p = 0.0198, Supplementary Material S3v; independent samples t-test  t27 = −2.216, p = 0.035, Supplementary Material S3w). There is no statistical difference between Bronze and Iron Age females for δ15N (independent samples t-test  t25 = 1.889, p = 0.070, Supplementary Material S3x), nor for males between periods (independent samples t-test  t27 = 1.867, p = 0.073, Supplementary Material S3y). However, nineteen individuals remain unsexed. This valuable demographic information, in addition to larger datasets per each demographic group, may alter δ13C value results between sexes, and also provide answers to questions concerning the age at which children were incorporated into a social stratification system (e.g., if they reflect more typical adult δ13C values74,75). No age- or sex-based tests were run by site, culture, or subperiod as there is significant incongruity in sample sizes between and among these categories, which would result in meaningless comparisons.

Discussion

Two key questions were posed in this research: 1) Is there evidence for nuanced dietary evolution from the Early Bronze Age to Early Iron Age, and 2) if so, what might this imply as concerns communication and trade in the later prehistoric GHP? Broadly speaking, the isotopic data presented here indicate a gradual shift in subsistence strategies from the Early Bronze to Early Iron Age, with evidence for subtle variation between cultures within epochs. In keeping with previous findings, near exclusive consumption of C3 plants remains characteristic of the Early Bronze Age58. Samples from both the Bronze and Iron Ages largely fall within δ13C values typical of C3 plant consumers, with a gradual increase in values over time. More specifically, the Late Bronze Age Piliny/Kyjatice samples are the first to exhibit as a whole less negative δ13C ratios, indicating a substantial shift from C3 plants or

Figure 5. Violin plot of human δ13C and δ15N ratios by cultures listed in roughly chronological order: PN (Proto-Nagyrév, n = 4), FZ (Füzesabony, n = 12), HFTZ (Hatvan or Füzesabony, n = 6), PLKY (Piliny or Piliny/Kyjatice, n = 14), PG (pre-Gáva or Gáva, n = 8), PS (Pre-Scythian/Mezőcsát, n = 4), and SA (Scythian/Vekerzug, n = 19). Center black dot represents mean; center black line represents distribution.
aquatic resources to C₃ plants, concomitant with archaeological evidence for increased millet consumption. Early Iron Age pre-Scythian (Mezőcsát) samples continue the Late Bronze Age trend by exhibiting enriched δ¹³C ratios, also in keeping with evidence for heavy reliance on millet at this time. The Bronze and Iron Age samples also exhibit less variable δ¹⁵N ratios than previous periods. The slightly higher δ¹⁵N values of the Bronze Age compared to Iron Age samples indicate greater reliance on protein in the former period. Subtle changes between subperiods until the Early Iron Age point to a gradual shift from a more terrestrial omnivorous diet, potentially with a low trophic level aquatic resource influence. Although changes were detected for both males and females among the Bronze and Iron Age periods for δ¹⁵N ratios, these differences are similar to those seen in the broader pattern between periods (Supplementary Material S3h and S3j). As the pattern of change between the Bronze and Iron Ages is not limited to one sex, this shift in food consumption can be interpreted as occurring among the entire population, as demonstrated by less negative δ¹³C values. These data thus provide evidence for nuanced dietary changes between the Early Bronze to Early Iron Ages. The implications for these findings are addressed below.

**Middle Bronze Age millet consumption.** Although there is scattered evidence that broomcorn millet was present in Europe (including present-day Hungary) from the Early Neolithic, directly radiocarbon dating of millet grains from sites in Central and Eastern Europe disputes its initial economic importance in the human diet, or as a foddering source prior to the Bronze Age. It is hypothesized instead that broomcorn millet may have been gradually incorporated into subsistence strategies from the Middle to Late Bronze Age. While the AMS ¹⁴C results of Filipović et al. challenge this, our smaller dataset continues to indicate its slower incorporation, at least in the GHP. Moreover, though millet has been radiocarbon dated to ~ 1600–1400 BCE at Fajsz 18 (Hungary), it is otherwise virtually undocumented archaeologically until the Late Bronze Age in the GHP. However, our data indicate the presence of millet, or a grain with comparable δ¹³C ratios, may have already begun in the Middle Bronze Age. Specifically, the Füzesabony yielded variable δ¹³C ratios that span both traditional terrestrial C₃ and C₄ ranges (Table 3). Our results are supported by other recent isotopic, radiocarbon, and archaeobotanical findings. For example, millet grains have been identified in Middle Bronze Age contexts in Moldova, from where it may have spread west up the Danube into the GHP along with other trade items. An isotopic analysis of the contemporaneous Trzciniec culture of Lesser Poland, it was posited that broomcorn millet may have been introduced to the region through cultural interaction with, or migration of, the Otomani–Füzesabony (and/or Tumulus) culture, as suggested by the exchange of culturally diagnostic prestige objects (e.g., beads, pins, amber, maces, ceramics). Additionally, broomcorn millet was dated to the Middle Bronze Age at Maszkowice (Poland) where the authors note ceramic and metal artefacts are similar to those recovered in the south Tisza valley within the Otomani–Füzesabony tradition.

A web of long-standing, long-distance trans-Carpathian exchange and communication networks appears to have often followed rivers and tributaries that connected the GHP north via the Vistula, Elbe, and Oder rivers towards the Baltic and North seas, east via the Tisza into Lesser Poland and Ukraine, and south via the Sava, Morava, and Vardar rivers towards the Aegean. Several of our Füzesabony samples derive from the site of Mezőzombor–Kőzségi temető, located near the central Tisza River at the confluence of the GHP and mountains. Another sample, radiocarbon dated to 1740–1440 cal BCE, is from Nagyrozvágy-Papdomb, located on the Bodrog River; the site also has bronze and gold artefacts. Located near rivers, these sites were ideally situated for trade. As noted earlier, increased socio-political and metallurgical complexity in the Carpathian Basin is evidenced by advanced trade networks and communal cemeteries associated with the Otomani–Füzesabony. Furthermore, potential links between the Füzesabony and the introduction of millet, to contemporaneous Middle Bronze Age cultures of Lesser Poland and Ukraine, have been posited and are corroborated by our data, and archaeological evidence for complex communication and trade networks at this time. However, fully establishing whether millet was adopted or dispersed by the Füzesabony through trade (e.g. as part of a network package from other areas), or by migrants directly introducing this crop to the local GHP population, requires additional genetic and radiocarbon data along with strontium and oxygen isotope approaches. Moreover, data from other Middle Bronze Age GHP cultures (e.g., Tumulus, in prep.), are needed to address whether millet consumption gradually intensified from the Middle Bronze Age in the GHP, as suggested by our results, or if, as posited by Filipović et al., it became an important crop from the outset.

Lastly, it must be noted that the elevated δ¹³C values of this period may also, at least in part, result from consumption of livestock that had been grazed on C₄ plants. However, this is a less parsimonious explanation given previous cultures from the same region ought then to also exhibit elevated δ¹³C values if they or their livestock consumed wild C₄-enriched plants. Moreover, δ¹³C values of fauna (~21.8 to ~19.4‰ with a mean value of ~20.6‰ ± 0.6‰ (1σ)), we previously obtained from the GHP, are consistent with terrestrial C₃ environment ranges. 

**Scythian subsistence economy.** In general, the Early Iron Age samples exhibit δ¹³C ratios suggestive of an increase in C₄ plants, though remain proportionally more C₃-based. However, the Early Iron Age Scythian (Vekerzug) samples specifically display greater variability, with the reappearance of more negative δ¹³C values, indicating some individuals consumed a mix of C₃ and C₄ cereals. They furthermore exhibit a reduction in δ¹⁵N values in comparison to Early Bronze Age populations and the pre-Scythian (Mezőcsát). This is potentially associated with increased sedentism in some Scythian groups, but greater reliance on pastoralism and thus C₄ plants, in others. Additionally, these samples were consistently different to many other cultures for δ¹⁵N.
Central Asia, data from Iron Age sites in Eurasia, East-Central Europe, and the GHP point to what appears to be a locally more complex scenario. Despite scarce evidence for Early Iron Age sites in the GHP, which corroborates nomadic pastoralism, recent archaeobotanical, pollen, and now stable isotope findings, challenge the perception of Scythian societies as defined by pastoral nomadism. They instead depict a more complex scenario in which certain groups were nomadic herders, while others engaged in mixed farming or agro-pastoralism, potentially also occupying more settled communities. For example, macrofossils of six-row barley and millet were recovered at Rákoskeresztúr-Újmajor in the Alföld, while pollen records dating to the Hungarian Early Iron Age allude to both the intensification of pastoralism and the continued importance of a mixed farming regime, alongside highly developed iron metallurgy and ceramic manufacture.

Although the Scythians were historically portrayed as a nomadic-pastoralist warrior class, particularly in Central Asia, data from Iron Age sites in Eurasia, East-Central Europe, and the GHP point to what appears to be a locally more complex scenario. Despite scarce evidence for Early Iron Age sites in the GHP, which corroborates nomadic pastoralism, recent archaeobotanical, pollen, and now stable isotope findings, challenge the perception of Scythian societies as defined by pastoral nomadism. They instead depict a more complex scenario in which certain groups were nomadic herders, while others engaged in mixed farming or agro-pastoralism, potentially also occupying more settled communities. For example, macrofossils of six-row barley and millet were recovered at Rákoskeresztúr-Újmajor in the Alföld, while pollen records dating to the Hungarian Early Iron Age allude to both the intensification of pastoralism and the continued importance of a mixed farming regime, alongside highly developed iron metallurgy and ceramic manufacture.

Diversification of local economies and adaptation to local environments has been posited based on archaeobotanical data from several Scythian sites in Central Asia, which point to a similarly heterogeneous subsistence economy as identified in our dataset. Archaeobotanical evidence for floodplain cereal cultivation of broomcorn millet and hulled barley has been recovered in Ukraine, as has that of wheat, barley, millet, and rye in central Asia and Russia. Recent isotopic evidence for cereal consumption in Scythian populations has also been reported for sites in Siberia and East Central Europe. For example, the urban Belšk (Ukraine) population was found to generally be composed of more sedentary agro-pastoralists who focused on millet cultivation, and it was also posited that millet and C₃ cereals may have composed a significant proportion of the diets of two Scythian communities of the Minusinsk and Tuva basins (Siberia), but that consumption of animals foddered on C₃ plants would isotopically mask their contribution. It remains to be established whether this is associated with increased sedentism. Given genetic evidence that Scythian groups were comprised of Late Bronze Age herders, farmers, and hunter-gatherers, further stable carbon, nitrogen and strontium isotopes of Early and Middle Iron Age Scythian populations from a dataset derived from diverse cemeteries, will also help to identify potentially heterogeneous lifeways within and between Iron Age cultures throughout the Carpathian Basin, and Eurasia at large.

Lastly, it must be noted that manuring affects the δ¹⁵N values of crops and their consumers with cattle manure altering ratios by +2 to 8‰, and pig manure by +15 to 20‰. Given humans, who consume mainly herbivorous animal protein, have an expected δ¹⁵N range of +8.5 to 12.5‰, those consuming manured cereals in a mixed plant- and animal-based diet should exhibit a concentrated range between +6 and 9‰. Accordingly, our study shows that δ¹⁵N ratios progressively decrease from the Early Bronze to the Early Iron Age, with a stabilization of values that are likely due to manured crop consumption. The vast majority of our Scythian samples fall within the manured crop consumption range, suggesting a subsistence strategy of some animal protein intake combined with manured crops. This indicates more uniform agricultural practices that resulted in more homogenized isotopic values.

Conclusions
The previously undetected nuanced differences we report here between the isotopic signatures of distinct cultures, and throughout the Early Bronze to Early Iron Ages, demonstrate that dietary evolution remains as complex and nonlinear as the cultural processes, and economic strategies with which it is entangled. The continued amalgamation of research that includes both multi-isotopic and varied archaeological approaches will help shed further light on local and trans-Carpathian subsistence and trade. Lastly, due to the fact that we cover a wide range of cultures throughout a large time frame, some sample sizes are small. Future studies should build upon our results with larger datasets to provide an even higher resolution analysis of the detected trends.

Material and methods
Stable carbon and nitrogen isotope analyses were conducted on bone samples from 74 human individuals spanning the Early Bronze to the Early Iron Age from the GHP micro-region and the adjacent Northern Mountain Range (Supplementary Material S2). Biological sex of adult individuals was determined based on flexure of the mandibular ramus, and dimorphic traits for the distal humerus, cranial and postcranial skeleton. Adults were aged according to standard methods for the ilium, pubic symphysis, and diaphyseal aspect of ribs, and according to obliteration of ectocranial sutures. Subadult age was estimated based on the ossification of apophyseal and epiphyseal joints, development of dentition, and diaphyseal long bone measurements.
Age grouping follows Martin and Saller\textsuperscript{114}. When possible, material was assessed for palaeopathological data (Supplementary Material S2).

Collagen extraction was performed at the University College Dublin Conway Institute (Dublin, Ireland) following a modified version of the Longin method\textsuperscript{115}, which can be found in detail elsewhere\textsuperscript{116–118}. Each sample was weighed to ~0.6 mg and placed into a tin capsule. Several samples were processed twice to assure repeatability. All samples were within the acceptable range of two standard deviations of each other\textsuperscript{38}. Samples were processed using a Thermo Finnigan DeltaPlus XL mass spectrometer. The accuracy and precision of the measurements, based on repeated measurements of two international laboratory standards USGS40 and USGS41, is $\pm 0.1\%$ (1σ) for $\delta^{13}C$ and $\pm 0.1\%$ (1σ) for $\delta^{15}N$. All carbon stable isotopic results are expressed as a delta ($\delta$) value relative to Vienna Pee Dee Belemnite (VPDB), and all nitrogen stable isotopic results as a delta ($\delta$) value relative to ambient air (AIR). Samples were assessed based on carbon and nitrogen content or weight (%). Acceptable %C ranges for modern mammalian bone collagen are between 15.3% and 47%, and for %N between 5.5% and 17.3%; samples falling outside those ranges were deemed inappropriate for analysis\textsuperscript{119}. Statistical analyses were performed to assess differences between time periods, demographic groups (i.e., age and sex), and cultures. Statistical analyses were not conducted on certain groups when the number of samples was too few to yield any meaningful analyses (n ≤ 4). Each group was checked for normality using a Shapiro–Wilk test, and equality of variance with Levene’s test, with a p < 0.050 as the statistical significance level. For pairwise comparisons among groups, t-tests (for normally distributed data), and Mann–Whitney U tests (for abnormally distributed data) were employed using p < 0.050 as the statistical significance level. When comparing multiple groups and to determine significant differences between them, one-way ANOVA and Kruskal–Wallis tests were employed for normally and abnormally distributed data, respectively. Post-hoc analyses were performed in cases of significance according to the normality of the data (Tukey’s, Mann–Whitney U, and Bonferroni tests). Statistical data were generated using R (v. 3.6.3\textsuperscript{120}) using the ggplot2\textsuperscript{121} package to generate figures.

**Ethics statement.** All necessary permits were obtained for the described study, which complied with all relevant regulations and ethical approval (Herman Ottó Múzeum, Miskolc; Dobó István Castle Museum, Eger; Hungarian National Museum, Budapest; Déri Museum, Debrecen; Budapest History Museum—Aquincum Museum and Archaeological Park, Budapest; Damjanich János Museum, Szolnok).

**Data availability**
All data generated or analysed during this study are included in this published article (and its supplementary information files).

Received: 28 March 2022; Accepted: 22 September 2022
Published online: 10 October 2022

**References**
1. Pécsi, M. & Sárfalvi, B. *The Geography of Hungary* (Corvina, 1964).
2. Sherratt, A. *Economy and Society in Prehistoric Europe: Changing Perspectives* (Princeton University Press, 1997).
3. Millsaukas, S. *European Prehistory: A Survey* (Springer, 2011).
4. Visy, Zs., Nagy, M., & Kiss, Zs. (eds.). *Hungarian Archaeology at the Turn of the Millennium* (Ministry of National Cultural Heritage & Teleki László Foundation, 2003).
5. Blaser, M. & Conrad, R. Stable carbon isotope fractionation as tracer of carbon cycling in anoxic soil ecosystems. *Curr. Opin. Biotechnol.* 41, 122–129. https://doi.org/10.1016/j.copbio.2016.07.001 (2016).
6. Chisholm, B., Nelson, D. & Schwar, H. Stable carbon isotope ratios as a measure of marine versus terrestrial protein in ancient diets. *Science* 216(4550), 1131–1132. https://doi.org/10.1126/science.1130.1132 (1991).
7. Ehleringer, J. & Monson, R. Evolutionary and ecological aspects of photosynthetic pathway variation. *Annu. Rev. Ecol. Syst.* 24, 411–439. https://doi.org/10.1146/annurev.es.24.110193.002211 (1993).
8. Fuller, B., Fuller, J., Harris, D. & Hedges, R. Detection of breastfeeding and weaning in modern human infants with carbon and nitrogen stable isotope ratios. *Am. J. Phys. Anthropol.* 129(2), 279–293. https://doi.org/10.1002/apa.20249 (2006).
9. Treasure, E. R., Church, M. J. & Gröcke, D. R. The influence of manuring on stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) in Celtic bean (*Vicia faba L*): Archaeobotanical and palaeodietary implications. *Archaeol. Anthropol. Sci.* 8, 555–562. https://doi.org/10.1007/s12520-015-0243-6 (2016).
10. O’Leary, M. Carbon Isotope fractionation in plants. *Phytochemistry* 20(4), 553–567. https://doi.org/10.1016/0031-9422(81)85134-5 (1981).
11. Tieszen, L. Natural variations in the carbon isotope values of plants: Implications for archaeology, ecology, and paleoecology. *J. Arch. Sci.* 18(3), 227–248. https://doi.org/10.1016/0305-4403(91)90063-U (1991).
12. van der Merwe, N. J. & Medina, E. The canopy effect, carbon isotope ratios and foodwebs in Amazonia. *J. Arch. Sci.* 18(3), 249–259. https://doi.org/10.1016/0305-4403(91)90064-V (1991).
13. Hobbie, E. A. & Werner, R. A. Intramolecular, compound-specific, and bulk carbon isotope patterns in C$_3$ and C$_4$ plants: A review and synthesis. *New Phytol.* 161(2), 371–385. https://doi.org/10.1111/j.1469-8137.2004.00970.x (2002).
14. Marino, B. D. & McElroy, M. B. Isotopic composition of atmospheric CO$_2$ inferred from carbon in C$_3$ plant cellulose. *Nature* 349, 127–131. https://doi.org/10.1038/349127a0 (1991).
15. 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.* 13(1–2), 46–53. https://doi.org/10.1002/oa.662 (2003).
16. Hobson, K., Barnett-Johnson, R., & Cerling, T. Using isoscapes to Track Animal Migration. In *Isoscapes: Understanding Movement, Pattern, and Process on Earth Through Isotope Mapping* (eds. West, J., Bowen, G., Dawson, T., & Tu, K.) 273–298 (Springer, 2010).
17. Schoening, M. J. & DeNiro, M. J. Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. *Geochim. Cosmochim. Acta* 48(4), 625–639. https://doi.org/10.1016/0016-7037(84)90091-7 (1984).
18. Nadelhoffer, K. & Fry, B. Nitrogen Isotope Studies in Forest Ecosystems. In *Stable Isotopes in Ecology and Environmental Science* (eds., Latjha, K. & Michener, R.) 22–44. (Blackwell, Oxford, 1994).
111. Bernert, Zs., Évinger, S. & Hajdu, T. New data on the biological age estimation of children using bone measurements based on
112. Schour, I. & Massler, M. The development of the human dentition.
113. Schinz, H. R. & Case, J. T.
114. Meindl, R. S. & Lovejoy, C. O. Ectocranial suture closure: a revised method for the determination of skeletal age at death.
115. Iscan, M. Y., Loth, S. R. & Wright, R. K. Age estimation from the rib by phase analysis: White males.
116. Iscan, M. Y., Loth, S. R. & Wright, R. K. Age estimation from the rib by phase analysis: White females.
117. Brooks, S. & Suchey, J. M. Skeletal age determination based on the os pubis: a comparison of the Acsadi-Nemeskeri and Suchey–
118. Bogaard, A. et al. Crop manuring and intensive land management by Europe's first farmers. PNAS 110(31), 12589–12694. https://doi.org/10.1073/pnas.1309918110 (2013).
119. Ambrose, S. Effects of diet, climate and physiology on nitrogen isotope abundances in terrestrial foodwebs. J. Am. Dent. Assoc. 130(9), 1153–1160 (1941).
120. R Core Team. R: A Language and Environment for Statistical Computing (R Foundation for Statistical Computing, 2020).
121. Wickham, H. ggplot2: Elegant Graphics for Data Analysis (Springer, 2016).

Acknowledgements
This research was conducted as part of the first author's PhD dissertation project. For the mass spectrometry analysis portion of this research, we would like to thank the University of Florida at Gainesville's Geological Sciences Department for processing the collagen samples. The authors would also like to thank Manel Prada for providing assistance in producing the map.

Author contributions
A.M.C. conceived of the study. A.M.C. performed the isotopic lab work with the aid of B.G. A.M.C. performed the formal analyses with the aid of B.G.: J.D., Z.B., A.C., P.C., L.D., A.E., M.H., A.H., Á.K., J.K., P.K., L.S., Z.K.Z., and K.S. provided skeletal materials and/or interpreted archaeological or anthropological information. J.D., Kr.Ki., T.S., and T.H. performed the osteological analyses. A.M.C., K.S.D.C., and T.S. performed and created all statistical analyses and plots. R.P. and T.H. supervised the study. A.M.C. wrote the manuscript with input from all co-authors, particularly K.S.D.C.
Funding
Open access funding provided by Eötvös Loránd University. The isotopic analyses were funded by the Irish Research Council Postgraduate Scholarship (GOIPG/2015/2275)(research.ie) held by AMC, and the Marie-Curie H2020-MSCA-IF-2015 (703373) held by BG. The physical anthropological work of TH, TSZ, KrK, and JD was supported by grant of the Hungarian Research, Development and Innovation Office (Project Number: FK128013).

Competing interests
The authors declare no competing interests.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-022-21138-y.

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