

Crop manuring and intensive land management by Europe's first farmers

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The spread of farming from western Asia to Europe had profound long-term social and ecological impacts, but identification of the specific nature of Neolithic land management practices and the dietary contribution of early crops has been problematic. Here, we present previously undescribed stable isotope determinations of charred cereals and pulses from 13 Neolithic sites across Europe (dating ca. 5900–2400 cal B.C.), which show that early farmers used livestock manure and water management to enhance crop yields. Intensive manuring inextricably linked plant cultivation and animal herding and contributed to the remarkable resilience of these combined practices across diverse climatic zones. Critically, our findings suggest that commonly applied paleodietary interpretations of human and herbivore $\delta^{15}\text{N}$ values have systematically underestimated the contribution of crop-derived protein to early farmer diets.

agriculture | prehistoric | husbandry | paleodiet

The spread of domesticated crops and livestock from western Asia to Europe was associated with profound social and ecological consequences, including demographic expansion and nucleation, new forms of ritual practice, and long-term increase in socioeconomic disparity (1). Although the significance of the agricultural transition is widely acknowledged, the nature of early European farming and its role in shaping ecological and social change has been explained in radically different ways (2, 3). Previous work on the ecological interpretation of archaeobotanical weed assemblages associated with charred crop remains in Neolithic central Europe has identified highly productive and disturbed soil conditions indicative of labor-intensive management (4). Interpretations of weed evidence in southern (5) and northern (6) Europe similarly suggest that early farmers invested considerable labor in the maintenance of long-established cultivation plots. Investigations of faunal assemblages indicate that small-scale intensive herding was the norm in various regions across Europe (7). Pasturing herds near hamlets and villages of early farmers would have enabled integration of cultivation and livestock keeping, with periodic stalling and use of crop material as fodder, introducing scope for use of manure on arable land (8). The practice of manuring to enhance soil productivity and tractability has implications for the long-term outlook of farmers on account of the “slow-release” of essential macronutrients (9). Intensive manuring, therefore, has important implications for “investment” in land and territorial claims by farming groups (10). Rarely preserved paleosols provide direct evidence of manuring with animal dung as far back as the Neolithic (11), but neither these sporadic indications nor the more indirect evidence of fodder remains, animal pens/byres, dung

accumulation at settlement sites, and “field scatters” around them reveal the intensity of manuring or its application to specific crops (5, 7–9).

Weed ecological approaches lack the resolution to identify manuring per se as a cause of high soil productivity; however, recent work on modern crops has shown that manuring with animal dung has a clear effect on stable nitrogen isotope ratios ($\delta^{15}\text{N}$ values) in cereals, the degree of impact depending on the intensity of manuring; that is, the level and frequency of application (12–14). Fig. 1 summarizes ranges of cereal $\delta^{15}\text{N}$ values for different manuring rates based on long-term agricultural experiments. In studies of real farming situations, manured plots typically encompass variable rates of manuring, resulting in variable cereal $\delta^{15}\text{N}$ values in the medium to high ranges (Fig. 1) (13). Pulses growing under the same conditions as cereals have lower $\delta^{15}\text{N}$ values because of atmospheric N fixation; only extremely intensive manuring (>35 tons/ha) results in clear elevation of pulse $\delta^{15}\text{N}$ values above 0‰ (13).

Significantly, manuring of cereal crops could elevate their $\delta^{15}\text{N}$ values above (unmanured) herbivore forage (e.g., woodland edge vegetation, leaf/branch fodder), so that humans eating manured crops would have $\delta^{15}\text{N}$ values higher than herbivores eating forage. Because offsets between human and herbivore $\delta^{15}\text{N}$ values in bone collagen are widely used to determine human trophic level (15), consumption of manured crops would enhance the apparent contribution of animal protein to the human diet. In the absence of crop nitrogen isotope determinations, paleodietary reconstructions based on $\delta^{15}\text{N}$ offsets between human and herbivore collagen at early farming sites in central and northwest Europe have likely overestimated consumption of animal protein at >70% (15).

Moreover, crop water status can be inferred from carbon isotope discrimination ($\Delta^{13}\text{C}$ values, which take into account changes in $\delta^{13}\text{C}$ of source CO_2 through time) (*Materials and Methods*) by reference to modern experimental and farm studies that have identified watering thresholds for wheat, barley, and pulses (16–18). Stable carbon isotope ratios in cereal grain and pulse seed are largely unaffected by manuring (*Materials and*

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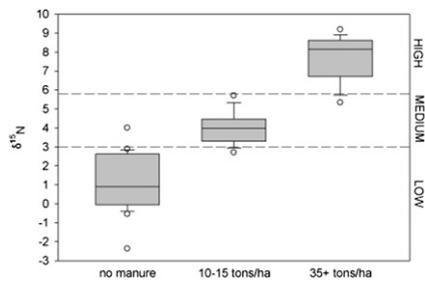


Fig. 1. Ranges of $\delta^{15}\text{N}$ values in modern bulk cereal samples (wheats and barleys) grown under different rates of manuring at long-term agricultural experiments: Rothamsted, United Kingdom, Askov, Denmark, and Bad Lauchstädt, Germany (13). Dashed horizontal lines represent thresholds of low (i.e., residual from previous land use history only), medium, and high manuring rates.

Methods) (18) and can be used in conjunction with crop $\delta^{15}\text{N}$ values to assess growing conditions and management practices, such as irrigation.

Herein we present unique evidence for crop growing conditions and farming practices based on carbon and nitrogen stable isotope determinations of 124 bulk crop samples (totaling >2,500 individual cereal grains or pulse seeds) from 13 Neolithic sites across Europe (Figs. 2 and 3), interpreted in the light of herbivore forage $\delta^{15}\text{N}$ values estimated from stable isotopic analysis of associated (domestic and wild) herbivore bone collagen, modern isotopic comparanda (12, 13, 16–18), experimental work on the effects of charring (19, 20), and associated arable weed assemblages (4, 5, 21, 22). The sites mostly date to the earlier Neolithic in their respective regions (Table 1) and were selected on the basis of richness in well-preserved charred crop material (Fig. 3); together, these sites provide a rough “transect” from southeast to northwest Europe (Fig. 2).

Results and Discussion

Fig. 4 shows $\Delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of cereals and pulses from four sites, and Table 2 summarizes results from all sites (for individual sample results, see Table S2). Unsurprisingly, a broad climatic trend in humidity is apparent if wheat or barley $\Delta^{13}\text{C}$ values at sites in or near the Mediterranean zone are compared with those in temperate zones (Fig. 2): thus, $\Delta^{13}\text{C}$ values of wheat

(or barley) from sites in Greece and Bulgaria are lower than those from Germany and Denmark (Fig. 4 and Table 2). Extensive isotope investigations of cereals and pulses from modern experimental stations and farm studies across Europe, however, have shown that crop $\delta^{15}\text{N}$ values reflect management practices rather than broad climatic trends (13). For six of the sites in Table 2, herbivore forage $\delta^{15}\text{N}$ values have been estimated from the $\delta^{15}\text{N}$ determinations of associated bone collagen of large herbivores by subtracting 4‰ as an average of the 3–5‰ range associated with the trophic shift (15). At these sites, cereal grain $\delta^{15}\text{N}$ values tend to be considerably higher than those inferred for forage. Although variation in $\delta^{15}\text{N}$ values among cereal components may contribute to this contrast (cereal grain is ca. 2.5‰ higher in $\delta^{15}\text{N}$ than associated rachis) (12, 13), even domestic herbivores receiving supplementary cereal fodder consumed a range of taxa and plant matter from various habitats (8) and, hence, inferred forage stable isotope values provide an approximation of the local $\delta^{15}\text{N}$ value of nonarable vegetation. Contrasts in cereal and forage $\delta^{15}\text{N}$ values suggest that arable soil N was relatively enriched in ^{15}N . Weed evidence available at a number of sites indicates that cultivation plots were long established, with soils tending to be intensively disturbed and productive (4, 5, 21, 22). Although these conditions exclude some potential causes of ^{15}N -enrichment, such as wetland denitrification, salinity, and recent clearance (32, 33), they constitute a plausible context for manuring.

Cereal grain $\delta^{15}\text{N}$ values at most sites are consistent with significant rates of manure application (Figs. 1 and 4, and Table 2). There is also considerable diversity in cereal $\delta^{15}\text{N}$ values at some sites. Local variation in manuring rates was observed in stable isotope studies of present-day traditional farming regimes in Asturias, Spain, and the Sighisoara region of western Romania (13). Variation is likely where manure is concentrated at specific locations in the landscape because it is heavy to transport and so tends to be used in close proximity to pens, byres, and so forth (9, 34). Low cereal $\delta^{15}\text{N}$ values (<3‰) indicative of long-term unmanured cultivation occur alongside high values at some sites (Fig. 4 A–C, and Table 2), and are dominant among the emmer wheat samples from Sarup, Denmark (Fig. 4D). Herbivore forage $\delta^{15}\text{N}$ values from all sites (ca. 1–3‰) (Table 2) fall within the range of unmanured cereals (Fig. 1).

Estimated forage $\delta^{15}\text{N}$ values are often similar to those of pulses. As expected (13), pulses tend to exhibit lower $\delta^{15}\text{N}$ values than associated cereals (Fig. 4 A and B, and Table 2). Only extremely intensive manuring (>35 tons/ha over a prolonged period, resulting in the formation of “dung-soil”), as observed in a study of present-day broad bean gardens in Evvia, Greece (13), increases pulse $\delta^{15}\text{N}$ values considerably above 0‰. The archaeobotanical pulse $\delta^{15}\text{N}$ values thus point to intensive manuring, variously comparable to, or higher than, the rates applied to associated cereals. Furthermore, high pulse $\Delta^{13}\text{C}$ values relative to wheat at sites in Greece and Bulgaria (Fig. 4 A and B, and Table 2) indicate a higher water status and suggest preferential use of well-watered soils for pulses or supplementary irrigation.

Overall, the crop nitrogen isotope compositions indicate that early farmers in Europe made strategic use of manure as a resource that was limited both by the scale of herding and by the



Fig. 2. Map showing the archaeological sites.



Fig. 3. Neolithic hulled barley grain from Koufouvouno.

underrepresent the importance of crop-derived protein (cf. 15). On the other hand, the findings presented here do not suggest a general geographical trend in crop $\delta^{15}\text{N}$ values that could

account for the relatively high human $\delta^{15}\text{N}$ values and large human-herbivore offsets often reported from sites in northwest Europe (15). That trend may in part reflect a reduction in the

Table 2. Summary of stable isotope determinations for archaeobotanical crop samples and associated herbivore bone collagen (n = number of samples)

Site	Category	n	$\delta^{13}\text{C}$		$\Delta^{13}\text{C}^*$		$\delta^{15}\text{N}^\dagger$	
			\bar{x}	σ	\bar{x}	σ	\bar{x}	σ
Koufovouno	Free-threshing wheat	12	16.5	0.5	-22.7	0.5	5.9	0.7
	Hulled barley	7	-24.5	0.3	18.4	0.4	2.7	1.2
	Pea	7	-24.9	0.9	18.6	0.8	1.3	0.3
	Large domestic herbivore	37	-20.5	0.7			4.9	1.0
	Large wild herbivore	1	-19.7				4.2	
	All large herbivore	38	-20.4	0.7			4.9	1.0
	Estimated herbivore forage [‡]					0.9		
Slatina	Einkorn wheat	6	-23.7	0.3	17.4	0.3	5.0	1.9
	Emmer wheat	6	-23.3	0.3	17.0	0.3	4.4	1.2
	Lentil	8	-24.9	0.2	18.6	0.2	1.7	0.6
	Grass pea	2	-24.0	0.0	17.8	0.1	1.5	0.3
	Large domestic herbivore	27	-20.3	0.6			6.5	0.7
	Large wild herbivore	5	-21.4	0.6			4.5	0.3
	All large herbivore	32	-20.5	0.7			6.2	1.0
	Estimated herbivore forage [‡]					2.2		
Kapitan Dimitriev	Einkorn wheat	1	-23.6		17.3		4.3	
	Emmer wheat	1	-23.6		17.3		3.8	
	Hulled barley	1	-23.4		17.1		5.5	
Azmak	Naked barley	1	-24.1		17.9		7.1	
	Pea	1	-23.9		17.6		2.9	
	Grass pea	1	-25.0		18.8		1.9	
Karanovo	Einkorn wheat	2	-23.8	0.1	17.6	0.1	3.6	0.4
	Emmer wheat	4	-22.9	0.1	16.7	0.1	3.3	0.2
	Lentil	2	-23.8	0.3	17.7	0.3	1.1	0.4
	Bitter vetch	1	-25.1		19.0		1.6	
Ecsegfalva	Emmer wheat	1	-23.9		17.6		5.7	
	Naked barley	3	-24.4	0.3	18.2	0.4	5.1	0.9
	Large domestic herbivore	19	-20.9	0.7			7.2	1.0
	Large wild herbivore	8	-20.9	0.5			7.0	1.0
	All large herbivore	27	-20.9	0.7			7.1	1.0
	Estimated herbivore forage [‡]					3.1		
Vaihingen	Einkorn	8	-24.1	0.4	18.2	0.5	4.6	0.4
	Emmer wheat	5	-24.0	0.6	17.9	0.6	4.2	0.6
	Lentil	1	-23.1		16.9		3.5	
	Pea	2	-23.8	0.2	17.7	0.2	2.4	1.9
	Large domestic herbivore	32	-22.5	0.9			6.8	0.8
	Large wild herbivore	11	-23.2	0.4			6.4	0.4
	All large herbivore	43	-22.7	0.8			6.7	0.7
	Estimated herbivore forage [‡]					2.7		
Hornstaad	Emmer wheat	2	-25.0	1.2	19.1	1.2	6.4	0.2
	Free-threshing wheat	9	-25.0	0.5	19.1	0.5	4.5	1.2
	Naked barley	6	-26.8	0.6	21.0	0.6	4.2	1.2
	Large domestic herbivore	7	-24.3	1.2			5.4	0.7
	Large wild herbivore	10	-24.3	0.8			4.8	0.5
	All large herbivore	17	-24.3	1.0			5.0	0.6
	Estimated herbivore forage [‡]					1.0		
Sarup	Emmer wheat	13	-24.6	0.4	18.7	0.5	2.5	0.9
Skaghorn	Emmer wheat	1	-23.0		17.1		6.1	
	Naked barley	1	-25.9		20.1		4.1	
Damsbo	Emmer wheat	1	-24.2		18.3		5.1	
Hambleton Hill	Emmer wheat	3	-23.1	0.7	17.2	0.7	3.6	0.6
	Large domestic herbivore	28	-21.4	0.5			5.1	0.6
	Large wild herbivore	5	-21.6	0.7			5.5	1.6
	All large herbivore	33	-21.5	0.6			5.2	0.8
	Estimated herbivore forage [‡]					1.2		
Lismore Fields	Emmer wheat	5	-23.4	0.1	17.4	0.1	5.4	0.3

*For calculation, see *Materials and Methods*.

[†] $\delta^{15}\text{N}$ values adjusted for charring by subtracting 1‰ (see *Materials and Methods*).

[‡]herbivore forage $\delta^{15}\text{N}$ estimate = herbivore mean minus 4‰.

diversity and dietary importance of pulse crops through central Europe, with pulses apparently dropping out of the crop spectrum altogether by the time agriculture reached Britain and Ireland in the early fourth millennium cal B.C. (6, 35). It also remains plausible that animal-derived protein generally increased in importance as farming spread into central and especially northern Europe (7). This inference is supported by recent detection of milk residues in specialized vessels for straining curds from the Kuyavia region of Poland dating back to the sixth millennium cal B.C. (36), and simulation modeling of the emergence of lactase persistence, which points to origins in Neolithic central Europe (37), although crops may often have remained the predominant protein source (38).

Conclusions

The crop stable isotope compositions presented here have important implications for the nature and role of early farming, and reveal distinct local or regional strategies in the use of manure. More broadly, the findings provide new insights into the system of relationships connecting Neolithic communities, their crops, livestock, and land. Neolithic farmers made investments, such as manuring with a long-term view to sustainability and territorial claims. This long-term outlook brings into focus a world view that gave rise to spectacular statements of permanence and ancestry, such as the funerary monuments of western Europe, as well as to extreme violence, evidenced by a mass burial of the late sixth millennium cal B.C. at Talheim, southwest Germany, where an entire community was killed by assailants wielding stone axes like those used to clear land (39). The fresh perspective on land use developed here lends support to the inference that differential access to intensively managed, heritable plots of land contributed to social differentiation in early European farming communities (40). Independent archaeobotanical evidence for the intergenerational transfer of arable land within kin groups is available at Vaihingen, southwest Germany (41). We hypothesize that manuring, like milking (42), dates back to the initial crystallization of the mixed farming package in western Asia and facilitated the spread of these entangled practices across diverse climatic zones in Europe.

Materials and Methods

Carbon isotope determinations of bulk crop samples (~200–300 cereal grains or 30–50 pulse seeds, both randomly selected) (13) from three long-term (>100 y) agricultural experiments show that $\delta^{13}\text{C}$ values are largely unaffected by manuring (Table S1). The ameliorating effect of manuring on water retention may be apparent in wheat samples from individual years of two short-term experiments, one at Sutton Bonington, United Kingdom and the other near Aleppo, Syria, but overall the experimental results indicate that manuring has no consistent effect on carbon isotopes in cereal grain and pulse seed (see also ref. 18).

Most archaeobotanical cereal grain samples (87 of 99) consisted of at least 10 whole grains from the same stratigraphic unit; in 12 cases two to eight grains were sampled because of limited availability of material. Most pulse samples (17 of 25) likewise consisted of at least 10 seeds from the same unit; in eight cases two to nine seeds were sampled. Crop samples mostly derived from visible concentrations (storage deposits) of grain/seed; at Hornstaad-Hörnle IA, where stored cereals were preserved as intact ears, each bulk sample consisted of grain extracted from individual cereal ears distributed among multiple houses (21). The grains and seeds selected were virtually undistorted morphologically (slight puffing only), resembling modern material charred experimentally at around 230 °C for a prolonged period (up to 24 h) (19). Samples were examined at 7–45 \times magnification for visible surface contaminants, such as adhering sediment or plant roots; these were removed by gentle scraping. Samples were weighed and placed in glass test tubes in preparation for an acid-base-acid pretreatment, a procedure commonly applied to charcoal and charred plant remains before radiocarbon and stable isotope analysis (43, 44) and considered appropriate for use on archaeobotanical remains (19). This three-step procedure consisted of: (i) treatment with 10 mL of 0.5 M HCl at 70 °C for 30–60 min, or until any effervescing ceased, and then rinsing in distilled water three times; (ii) treatment with 10 mL of 0.1 M NaOH at 70 °C for 60 min, followed by rinsing in distilled water until the solution was clear and the pH neutral, using a minimum of three rinses; (iii) treatment with 10 mL of 0.5 M HCl at 70 °C for 30–

60 min, followed by three rinses in distilled water and final freeze drying. The initial acid step is applied to remove nonstructural carbonates, the base step to remove humic acids and the final acid repeat to remove CO_2 that can be incorporated during step two. Dried tissues were ground to fine homogeneous powder using a mortar and pestle and weighed into tin capsules for stable isotope analysis.

For faunal sampling, the humerus was selected (where possible) as the most common long bone across the major taxa. The distal end was targeted to enable broad age assessment. Samples of ~0.5–1 g were obtained from each specimen. Bone surfaces were cleaned of any visible dirt or carbonate crusts using an aluminum oxide air abrasive. Collagen was isolated using a modified gelatinization method based on the methods of refs. 45 and 46. Bone collagen samples of ~0.75 mg were weighed into tin capsules for stable isotope analysis.

Stable isotope analyses of bone collagen for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, plant $\delta^{15}\text{N}$ values and %C and %N contents were conducted on a ThermoFinnigan Flash 1112 elemental analyzer connected on-line to a Delta+XL isotope ratio mass spectrometer at the British Geological Survey, Keyworth, United Kingdom. The analysis of plant $\delta^{13}\text{C}$ values and %C contents was performed by combustion in a Costech 4010 on-line to a VG TripleTrap and Optima dual-inlet mass spectrometer. All $^{13}\text{C}/^{12}\text{C}$ ratios were calculated as $\delta^{13}\text{C}$ values on the Vienna Pee Dee Belemnite (VPDB) scale using within-run laboratory standards for bone collagen and plant material calibrated against NBS-19 and NBS-22. The $^{15}\text{N}/^{14}\text{N}$ ratios were calculated as $\delta^{15}\text{N}$ values versus atmospheric N_2 by comparison with laboratory standards for plant and bone collagen material calibrated against IAEA-N-1 and N-2. The relative analytical errors (1 SD) for replicate analytical standards were $\pm 0.2\text{‰}$ for $\delta^{13}\text{C}$ and $\pm 0.4\text{‰}$ for $\delta^{15}\text{N}$. Replicate analyses of bone collagen sample VAH35 measured in 11 separate mass spectrometry runs had a SD (1 SD) of $\pm 0.3\text{‰}$ for $\delta^{13}\text{C}$, $\pm 0.2\text{‰}$ for $\delta^{15}\text{N}$, and ± 0.2 for the C:N ratio (mean C:N of 3.3). Collagen yields ranged from 0.9 to 17.0% and the C:N ratios between the 2.9 and 3.6, which is within the accepted range for well preserved collagen (47). For plant material, the precision (1 SD) among replicates of a well-homogenized modern uncharred barley sample was 0.2 for %N and 0.4‰ for $\delta^{15}\text{N}$ analyzed in 29 separate runs and 3.5 for %C and 0.1‰ for $\delta^{13}\text{C}$, analyzed in 21 separate runs. New stable carbon and nitrogen isotope determinations of *Ecesgalva* fauna were considered together with previously published values (25). Herbivore collagen determinations from Hambledon Hill were published previously (29).

Changes in the atmospheric concentration of CO_2 throughout the Holocene are reported not to affect the interpretation of stable carbon isotope ratios (16, 48–50). However, changes in the $\delta^{13}\text{C}$ of the carbon component of atmospheric CO_2 must be taken into account. The $\delta^{13}\text{C}$ value of atmospheric CO_2 can be reconstructed from ice-core bubbles, and has decreased from ca. -6‰ , around 10,000 y ago (51, 52), to ca. -8‰ today (53). The $\delta^{13}\text{C}$ of atmospheric CO_2 for any given time-period can be approximated by the AIRCO2_LOESS system (54). This approach was used to calculate ^{13}C discrimination independent of source CO_2 (expressed as $\Delta^{13}\text{C}$) (55).

Fig. S1 shows that the C:N ratios of the archaeobotanical crop specimens resemble those of modern cereal grains and pulse seeds experimentally charred under conditions replicating ancient morphologically intact specimens (at 230 °C for 24 h under a reducing atmosphere), and as expected the pulse samples are higher in %N than the cereals (19). Biomolecular assessments of modern cereal grains and pulse seeds charred under these conditions suggest that original $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values are reliably retrievable (19, 20).

Table S2 lists all of the archaeobotanical samples analyzed and gives their $\delta^{13}\text{C}$, $\Delta^{13}\text{C}$, and $\delta^{15}\text{N}$ values, along with %C, %N, and C:N. The $\delta^{13}\text{C}$ values of cereal and pulse crops range from -27.8 to -22.1‰ , and are typical for C_3 plants in Europe. To account for the observed effects of experimental charring (at 230 °C for 24 h under a reducing atmosphere) on cereal grain and pulse seed $\delta^{15}\text{N}$ values (19, 20), 1‰ is subtracted. This adjustment is generous in light of observations that charring for shorter time periods does not bias cereal $\delta^{15}\text{N}$ (56) and, notably, works against both the manuring and paleodietary arguments developed here.

We estimated herbivore forage $\delta^{15}\text{N}$ values by subtracting 4‰ from mean herbivore $\delta^{15}\text{N}$ values (Table 2). A trophic shift of 4‰ is within the commonly used range of 3‰ to 5‰ (15).

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