



Further investigation into the impact of manuring on stable carbon ($\delta^{13}\text{C}$) and nitrogen isotope ($\delta^{15}\text{N}$) values in pulses: a four-year experiment examining Celtic bean (*Vicia faba*)

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Abstract

Plant stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope values can be used to directly investigate crop husbandry practices such as manuring; a key variable in understanding the scale and intensity of past farming practices. We present new results from a four-year experiment examining the impact of manuring on the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of a heritage cultivar of the broad or faba bean (*Vicia faba*), the ‘Celtic black broad bean’. This paper builds upon our previous pilot study (Treasure ER, Church MJ, Gröcke DR (2016) The influence of manuring on stable isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) in Celtic bean (*Vicia faba* L.): archaeobotanical and palaeodietary implications. *Archaeol Anthropol* 8:555–562. <https://doi.org/10.1007/s12520-015-0243-6>). Our results indicate that manuring increased $\delta^{15}\text{N}$ values in beans, with a mean value of $0.5 \pm 0.4\%$ in control samples compared to a mean value of $2.1 \pm 1.3\%$ in the most intensively manured plots. It was not always possible to distinguish between an unmanured and manured crop on the basis of $\delta^{15}\text{N}$ values, although manured crops consistently exhibited larger variation in $\delta^{15}\text{N}$. Bean $\delta^{13}\text{C}$ values show no clear relationship with manuring, although large variability ($> 3\%$) was found within crops cultivated under the same conditions. Manuring also increased plant biomass production, seed-size and the bean per plant ratio. Overall, we suggest that high $\delta^{15}\text{N}$ values ($> 1.5\%$) in archaeobotanical remains of pulses can be attributed to small-scale, intensive cultivation that is typical of gardens where manuring rates are very intensive.

Keywords Stable isotopes · Nitrogen · Carbon · Manuring · Pulses · *Vicia faba* · UN SDG15 “Life on land”

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Introduction

The scale and intensity of past crop husbandry practices is closely linked to wider research questions examining settlement patterns, land tenure, labour organisation and long-term soil health (Bogaard 2005; Jones 2005; Halstead 2014; Bogaard et al. 2013, 2016; Gron et al. 2021). Manuring is a key factor in understanding the nature of agricultural regimes since very high rates of manure application tend to be associated with small-scale and intensive cultivation practices (e.g., hoeing, weeding, watering, manuring) in plots near to settlements where animal and crop husbandry are closely integrated (Bogaard 2005). *Intensive* cultivation regimes are defined by high labour inputs per unit area of land, as opposed to *extensive* cultivation where labour per unit area are low and field sizes tend to be larger (Morrison 1994). Despite their small size, the produce from small, intensively cultivated plots (akin to gardens) can contribute substantially to the diets of subsistence farmers and they can provide key sources of income in market economies, with manuring being essential to maintain fertility and yields (Grigg 1974:125–128; Netting 1993).

Manuring can be identified in plants through examination of nitrogen isotope ratios (expressed in $\delta^{15}\text{N}$ values). It is well-established that applications of animal manure and other organic fertilisers (e.g., seaweed, fish, bonemeal) increase the $\delta^{15}\text{N}$ value of soils and plants (for review, see Szpak 2014). At present, most research has examined cereals such as wheat (*Triticum* spp.) and barley (*Hordeum* spp.), with experiments and farm studies indicating clear and consistent ^{15}N -enrichment in grain and chaff as a result of manuring (Bogaard et al. 2007; Kanstrup et al. 2011; Fraser et al. 2011). Variation in cereal $\delta^{15}\text{N}$ values depends on manuring rates, with intensive applications of cattle manure (~35 t/ha) producing high $\delta^{15}\text{N}$ values of $> +6\%$ (Fraser et al. 2011). Additional information on cultivation conditions is provided by crop $\delta^{13}\text{C}$ values, which are strongly influenced by water availability (or soil moisture) in semi-arid/arid environments, although in temperate environments other factors (e.g., shade, soil type, temperature) may be more significant (cf. Gron et al. 2021; Bishop et al. 2022). Overall, numerous studies provide a basis for the interpretation of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in cereals and the identification of manuring. However, the impact of manuring on $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values in pulse crops has been examined in less detail and there remain gaps in current research.

Previous work suggests that manuring may only be identifiable in pulses where application rates are extremely high (Fraser et al. 2011; Treasure et al. 2016). This is because pulses are harvested from leguminous plants which are capable of obtaining a high proportion of nitrogen via atmospheric N_2 -fixation, whereas cereals (non- N_2 -fixers) obtain

nitrogen from the soil (Unkovich and Pate 2000). N_2 -fixing plants typically have $\delta^{15}\text{N}$ values of $0\% \pm 1\%$, reflecting the isotopic composition of atmospheric N (i.e., $\delta^{15}\text{N}_{\text{air}} = 0\%$) (Unkovich 2013). Theoretically, manuring may cause ^{15}N -enrichment in pulses (i.e., $> 1\%$) since high soil mineral content suppresses atmospheric N_2 -fixation rates (Unkovich and Pate 2000). However, this has yet to be examined in detail, with previous work suggesting that only extremely intensive manuring (i.e., > 35 tons/ha) elevates pulse $\delta^{15}\text{N}$ values (Fraser et al. 2011; Treasure et al. 2016). Equally, manuring is a potential source of variation in pulse $\delta^{13}\text{C}$ values since changes in plant biomass production, soil fertility and soil conditions (e.g., organic content) may indirectly influence water availability and plant water use (Cernusak et al. 2013). Manure application rates which are thought to significantly increase pulse $\delta^{15}\text{N}$ values are therefore only likely to be achieved in small, intensively cultivated plots akin to gardens.

Building upon data produced by Fraser et al. (2011), we previously undertook a pilot-study examining the impact of manuring on pulse $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values on a heritage cultivar of broad or faba bean (*Vicia faba*), the ‘Celtic black broad bean’ (Treasure et al. 2016). Our initial results from one growing season, demonstrated manuring rates of approximately > 70 t/ha increased $\delta^{15}\text{N}$ values (up to $2.6\% \pm 0.3\%$), with potentially large variation in $\delta^{13}\text{C}$ values (up to 1.7%). Manuring also increased the size of beans. In this paper, we have expanded our study and present data from a four-year experiment to examine in greater detail the relationship between manuring intensity, plant biomass, seed-size and stable isotope ratios ($\delta^{15}\text{N}$, $\delta^{13}\text{C}$) in Celtic black broad bean. Our key research aim was to assess if there is a consistent, multi-seasonal isotopic response to intensive soil amendment for a pulse crop, specifically addressing to what extent manuring influences a) biomass production and seed size and (b) $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values. *Vicia faba* is one of the most commonly recovered pulses in archaeobotanical assemblages across the Near East, North Africa and Europe from the Neolithic and Bronze Age onwards, becoming especially common during the Roman and medieval periods (College and Conolly 2007; Zohary et al. 2012; Stika and Heiss 2013; Treasure and Church 2017). We suggest that this research is likely to be applicable to a range of other pulse crops, with potential to provide insights into the nature of past manuring practices, and by extension the scale and intensity of cultivation regimes.

Methods

Experimental design

The four-year experiment (2014–2016, 2018) was undertaken in ten 1-m² outdoor plots at Durham University Botanic Gardens using a heritage variety of faba/broad bean, the ‘Celtic black broad bean’. 1-m² plots were specifically chosen to simulate intensive ‘garden cultivation’ practices that have been proposed for pre-Industrial north-west European pulse cultivation for thousands of years (Bogaard 2005; Jones 2005). Exact replication of past growing conditions has not been attempted; instead, an experimental simulation approach was followed to inform the interpretation of the archaeological record (see Outram 2008). Celtic black broad bean produces small, rounded seeds which are morphologically similar to prehistoric and later finds of *Vicia faba* var. *minor* (Neef et al. 2012; Zohary et al. 2012; Treasure and Church 2017).

The ten 1-m² plots comprised unmanured ‘control’ plots ($n=4$) and manured plots ($n=6$), with two manuring rates: Soil Amendment Treatment – 1 (‘SAT-1’) and Soil Amendment Treatment – 2 (‘SAT-2’). In 2014–2016, ‘SAT-1’ ($n=4$) consisted of composted farmyard manure (horse/cattle manure, cereal straw) and ‘SAT-2’ ($n=2$) consisted of pure sheep dung, mixed with composted leaf litter and domestically produced compost (containing no animal products). The plot was left fallow in 2017. In 2018, both amended plots (SAT-1 and SAT-2) received pure sheep dung mixed with composted leaf litter since the original composted farmyard manure was not available. Hazel wood ash (0.25 L) was added to the amended plots to simulate the addition of hearth debris, a common amendment strategy in past cultivation practices (cf. Guttman et al. 2008). Details of the amendment/manuring methods are outlined in Table 1. The rates of amendment application (SAT-1 plots ~70 t/ha; SAT-2 plots ~150 t/ha) reflect approximations only. Sowing was undertaken in April/May, with 10 beans planted in each plot, apart from 2014 when 20 beans were planted (see Supplementary Material for details). The beans had been soaked in water for 48 h to initiate germination. A single weeding of each plot was undertaken in July of each year. Whole plants were harvested in September at

the same time and plant biomass recorded for plants that had not suffered significant pest damage (plant height, pod/bean number, bean dimensions measured using a Mitutoyo ABS Digimatic Calliper). In 2018, only a proportion of the beans were measured (852 out of 4209 harvested) due to the very high numbers produced that year, however, the total beans produced from each plant were fully counted and these are included in the summary metrics in Table 2.

Stable isotope analysis

Eight beans per treatment type were randomly selected for isotope analysis, avoiding possible duplicate sampling of beans from the same plant. All the beans from a plot from a particular harvest were sequentially numbered and then randomly chosen using a true random number generator: www.random.org (Haahr 2024). Only beans were chosen for isotope analysis, as they are usually the only plant part found in the archaeological record (Treasure and Church 2017). This corresponds to 24 single-entity samples per year: control plots ($n=8$; 2 from each plot), SAT-1 plots ($n=8$; 2 from each plot), SAT-2 plots ($n=8$; 4 from each plot). Individual beans were homogenised, then weighed into tin capsules for stable isotope analysis.

Carbon and nitrogen isotope analysis of the bean samples were performed using a Costech Elemental Analyser (ECS 4010) connected to a Thermo Scientific Delta V Advantage isotope ratio mass spectrometer in the Stable Isotope Biogeochemistry Laboratory (SIBL) at Durham University. Isotopic accuracy was monitored through repeated analyses of international and in-house standards (see Gröcke et al. 2021). Analytical uncertainty in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ was <0.1‰ (2 sd) for replicate analyses of a range of international standards analysed each day ($n=8$ different types, e.g., USGS40, USGS24, IAEA-600, IAEA-CH-3, IAEA-N-1, IAEA-N-2, NBS19, IAEA-CH-6) and <0.2‰ (2 sd) for in-house standards ($n=4$ different types: sugar, cellulose, urea and glutamic acid) and replicate sample analysis. Total organic carbon and nitrogen data was obtained as part of the isotopic analysis using the in-house standard glutamic acid (carbon = 40.82‰, nitrogen = 9.52‰). Nitrogen isotopic composition of the manure used was previously

Table 1 Plot and treatment types, indicating approximate amendment application rates

Plot type	Treatment Per Plot	Approximate manuring rate
Control	No manure applied	
Soil Amendment Treatment 1 (SAT-1)	2014–2016: 15 L (~7 kg) of composted farmyard manure (cattle/horse dung, straw) mixed with compost/leaf litter + 0.25 L of hazel wood ash. 2018: 14 L (~8 kg) of pure sheep dung mixed with compost/leaf litter + 0.25 L of hazel wood ash.	7–8 kg/m ² (70–80 t/ha)
Soil Amendment Treatment 2 (SAT-2)	2014–2016: 14 L (~8 kg) of pure sheep dung and with 15 L (~7 kg) of composted farmyard manure (cattle/horse dung, straw) mixed with compost/leaf litter + 0.25 L of hazel wood ash. 2018: 28 L (~16 kg) of pure sheep dung, mixed with compost/leaf litter + 0.25 L of hazel wood ash.	15–16 kg/m ² (150–160 t/ha)

Table 2 Summary of plant biomass results and stable isotope results combined for the four-year experiment. Further summary tables and the raw data are presented in the electronic supplementary material. The compact letter display attributes a shared group letter between any two groups for which there is no evidence of a difference between their group means (Piepho 2004). *Average bean production per plant estimated by dividing the number of beans by the number of plants

		Manured plots		
		Control	SAT-1	SAT-2
Biomass results				
Number of plots (total)		16	16	8
Number of plants		154	151	65
Number of pods		602	929	510
Number of beans		1809	2803	1638
Bean per plant*		12	19	25
Plant height (cm)		66 ± 19 ^a	83 ± 21 ^b	102 ± 20 ^c
Bean dimensions (mm):				
	Length	10.42 ± 1.48 ^a	10.90 ± 1.84 ^{ab}	10.63 ± 1.44 ^b
	Width	7.47 ± 1.01 ^a	7.69 ± 1.16 ^a	7.27 ± 0.98 ^a
	Depth	8.61 ± 1.26 ^a	8.95 ± 1.51 ^a	8.56 ± 1.22 ^a
Stable isotope results				
Number of samples		32	32	32
$\delta^{15}\text{N}$ (minimum)		-0.4	-0.5	0.8
$\delta^{15}\text{N}$ (maximum)		1.5	5.9	6.0
$\delta^{15}\text{N}$ (mean, 1 sd)		0.5 ± 0.4 ^a	1.1 ± 1.6 ^a	2.1 ± 1.3 ^b
$\delta^{13}\text{C}$ (minimum)		-32.4	-32.2	-33.9
$\delta^{13}\text{C}$ (maximum)		-27.9	-26.1	-27.0
$\delta^{13}\text{C}$ (mean, 1 sd)		-30.4 ± 1.0 ^a	-29.9 ± 1.3 ^a	-30.4 ± 1.5 ^a

determined: farmyard manure $\delta^{15}\text{N} = +7.7\text{‰}$, and sheep dung $\delta^{15}\text{N} = +7.5\text{‰}$ (Treasure et al. 2016).

Statistical analysis

To support the observational results, we carried out statistical tests to compare the pairwise mean differences of each measured variable across the three groups (Control, SAT-1, SAT2). Levene's test for homogeneity (Levene 1960; Fox 2015) provided evidence of heterogeneity across groups for three of these variables, with p-values below 0.05. As a result, we utilised the Tukey pair-wise comparison T-tests between all three groups based on a generalised linear model for each variable of interest, with soil type as regressor (all groups surpassing $n = 32$, justifying the central limit theorem). The compact letter display attributes a shared group letter between any two groups for which there is no evidence of a difference between their group means (Piepho 2004).

Results

Table 2 summarises average plant biomass and stable isotope results ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) for each year. Figure 1 presents $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for each year, whilst Fig. 2 presents mean $\delta^{15}\text{N}$ values for the four-year period. The electronic supplementary material contains the full dataset for each plot and year, including biomass results and stable isotope results ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, C/N ratio, weight% C and % N).

Plant biomass, seed number and size

In total, 370 plants were harvested over the 4 seasons, producing 6250 beans, of which 2893 beans were measured for length, width and depth (Table 2). The biomass measurements from the 3 treatment types have been combined from all of the plots over the 4 seasons in Table 2 to summarise the general picture from the data. A more detailed breakdown by plot and by year is presented in the Supplementary Material, demonstrating the variability between each plot by treatment and time. Overall, plants cultivated in manured plots exhibit higher bean-per-plant yields and plant biomass production increased with amendment (using plant height as a proxy for biomass). Seed-size varies widely between years and treatment type with mean values for the four-year period indicating increases in bean length, width and depth in SAT-1 samples, whilst only bean length increased in SAT-2 samples. Due to the very large sample size, and the measurement of the entire populations in 2014, 2015 and 2016, the mean values and associated standard deviations provide accurate reflections of variation in bean size.

Carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope values

Mean $\delta^{13}\text{C}$ values over the four-year period are $-30.4\text{‰} \pm 1.0\text{‰}$ in control samples, $-29.9\text{‰} \pm 1.3\text{‰}$ in SAT-1 and $-30.4\text{‰} \pm 1.5\text{‰}$ in SAT-2 samples. Within plot variation in $\delta^{13}\text{C}$ is typically minimal ($< 1\text{‰}$), however, there was some large variation recorded in some plots (e.g., $> +3\text{‰}$, 2018 control plot 2) and within single treatment types (e.g., $> +4\text{‰}$, 2016 SAT-1 samples).

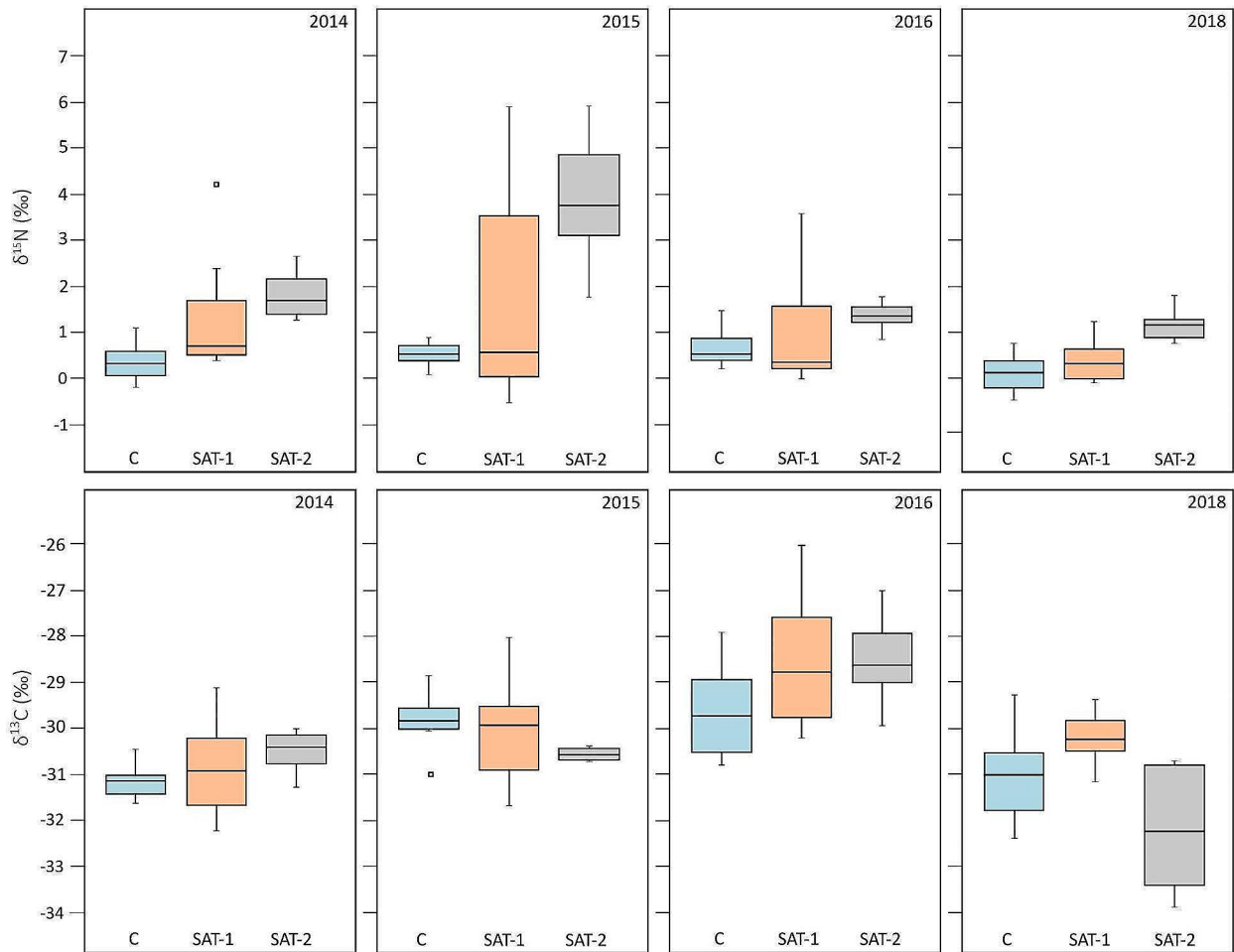


Fig. 1 Box and whisker plots displaying $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values for each year of the experiment. The whiskers reflect the minimum/maximum values (excluding outliers, defined as $1.5 \times$ the IQR) and the boxes

reflect the IQR, with the horizontal line in the boxes indicating the median. C: control. SAT-1 and SAT-2: see Table 1 for definitions

Mean $\delta^{15}\text{N}$ values over the four-year period are $+0.5\text{‰} \pm 0.4\text{‰}$ in control samples, $+1.1\text{‰} \pm 1.6\text{‰}$ in SAT-1 and $+2.1\text{‰} \pm 1.3\text{‰}$ in SAT-2 samples. The combined average $\delta^{15}\text{N}$ value of the SAT-1 and SAT-2 samples is $+1.6\text{‰} \pm 1.5\text{‰}$.

Control sample $\delta^{15}\text{N}$ values range from -0.4‰ to $+1.5\text{‰}$, with three samples having $\delta^{15}\text{N}$ values $> +1\text{‰}$. The SAT-1 samples range from -0.5‰ to $+5.9\text{‰}$, with large inter-annual and inter-plot variation (up to $> +6\text{‰}$). For example, in 2015 the SAT-1 plots have $\delta^{15}\text{N}$ values which are consistently $< +2\text{‰}$, with the exception of Plot 1 where individual samples have very high $\delta^{15}\text{N}$ values ($+5.2\text{‰}$, $+5.3\text{‰}$). Large variation in $\delta^{15}\text{N}$ values is also evident in the SAT-2 plots, which range from $+0.8\text{‰}$ to $+6.0\text{‰}$, with the highest $\delta^{15}\text{N}$ values similarly recorded in 2015 ($+5.4\text{‰}$, $+6.0\text{‰}$). Whilst the control sample $\delta^{15}\text{N}$ values follow a normal distribution, both the SAT-1 and SAT-2 sample $\delta^{15}\text{N}$

values have a non-normal and negatively skewed distribution. Consequently, in the SAT-1 and SAT-2 plots there is a large difference between the mean ($+1.1\text{‰}$, $+2.1\text{‰}$) and median ($+0.6\text{‰}$, $+1.5\text{‰}$) $\delta^{15}\text{N}$ values respectively.

Statistical results

The compact letter display groupings resulting from Tukey's pairwise comparison T-tests for each of the variables is presented in Table 2 as superscripts (a, ab, b, c). These results indicate that there is no evidence of difference in mean depth and $\delta^{13}\text{C}$. There is evidence that mean plant height differs between each of the groups, with each of the pairwise comparisons for this variable yielding p-values < 0.0001 . There is evidence of difference in bean length between Control and SAT-2, with a p-value of 0.0484. However, there

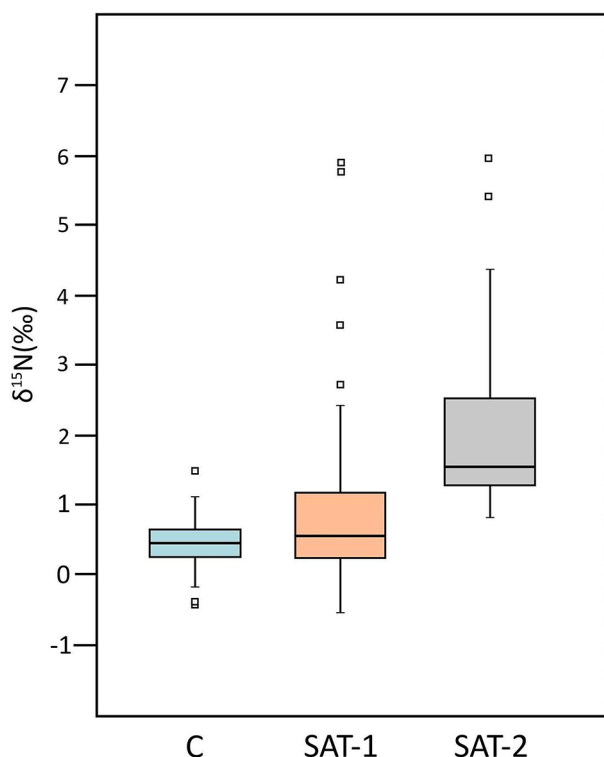


Fig. 2 Box and whisker plots displaying $\delta^{15}\text{N}$ values combined across the four-year experiment. The whiskers reflect the minimum/maximum values (excluding outliers, defined as $1.5 \times$ the IQR, which are plotted individually). The boxes reflect the IQR, with the horizontal line in the boxes indicating the median. C: control. SAT-1 and SAT-2: see Table 1 for definitions

is no evidence of difference in length between SAT-1 and either Control (p -value 0.0738) or SAT-2 (p -value 0.9216).

For variable $\delta^{15}\text{N}$, the pairwise t -test between control and SAT-1 yielded a p -value of 0.0703, indicating that there was no evidence that the means of these two groups were different. SAT-2 was placed in a distinct group, indicating that the pairwise t -test provides evidence that SAT-2 has a different mean to SAT-1 (p -value 0.0315) and control (p -value < 0.0001). In addition, for $\delta^{15}\text{N}$, a standard t -test was performed between the control group and the combined SAT-1 and SAT-2 groups (i.e. treated groups), with a resulting p -value < 0.0001 , thus providing strong evidence of a difference in mean values.

Discussion

Plant biomass, seed number and size

The beneficial effect of manuring is indicated by increased plant biomass production and higher bean production. Equally, manuring typically increased seed-size, especially length (confirmed by the Tukey test, see Table 2), although

inter-annual variation is apparent. Therefore, in the archaeological record, there should be a positive correlation between seed-size and $\delta^{15}\text{N}$ values provided that sample size is sufficiently large (i.e., larger than the dataset analysed here for stable isotopes). By measuring archaeobotanical pulse seeds, information on cultivation conditions may be obtained, with the expectation that larger seeds could reflect cultivation in more fertile and/or manured plots. Seed-size has, however, also been demonstrated to vary in relation to water availability and possibly also with planting/sowing depth (Fuller and Harvey 2006; Fuller 2007; Caracuta et al. 2015). Despite this, our results suggest that agricultural practices such as manuring (or cultivation on fertile soils) could be an important factor in understanding seed enlargement in pulses following domestication (cf. Fuller 2007). We therefore recommend that the dimensions of pulses and cereal grains are routinely measured prior to single-entity isotopic analysis (cf. Bishop et al. 2022) to maximise the interpretive value of the archaeobotanical research. This also highlights the future potential for using geometric morphometrics in combination with isotope studies (cf. Roushannafas et al. 2022).

Manuring as a source of variation in $\delta^{13}\text{C}$ values

In C_3 plants, water availability and temperature have a strong influence on ^{13}C -discrimination during photosynthesis, particularly in semi-arid/arid environments where water can be a significant limiting factor on plant growth (e.g., O'Leary 1981; Farquhar et al. 1982). However, several factors may directly or indirectly cause variation in water availability, such as soil nutrients, the physical characteristics of soils (e.g., organic content, sand vs. clay content) and biomass production (Cernusak et al. 2013). Manuring is therefore a potential source of variation in $\delta^{13}\text{C}$ values.

Our results indicate that manuring typically only caused small variation in $\delta^{13}\text{C}$ values ($< 1\%$), although this value masks large variation ($> 3\%$) seen in individual samples cultivated under the same conditions. No consistent directional change was observed between treatment types, confirmed by the Tukey tests between the groups (see Table 2). The cause of this variability is unclear, with no apparent correlation with rainfall or temperature (see Supplementary Material, Fig. 3). Previously, Gröcke et al. (2021) indicated that the carbon isotope composition of the soil may also have an effect on the $\delta^{13}\text{C}$ of crops growing on amended soils.

^{15}N -enrichment in manured pulses

High $\delta^{15}\text{N}$ values (i.e., $> 1\%$) were observed in SAT-1 and SAT-2 samples, with higher ^{15}N -enrichment in the more intensively manured SAT-2 samples, confirmed by

the statistical analysis presented in the Results (Table 2). This equates to a manuring rate of 70–150 t/ha, or 7–15 kg/m², although it should be emphasised that this is only an approximation. Individual samples exhibit spurious $\delta^{15}\text{N}$ values: some control samples ($n=3$) have ‘high’ $\delta^{15}\text{N}$ values (up to +1.5‰), whereas 53% of SAT-1 samples ($n=13$) have ‘low’ $\delta^{15}\text{N}$ values (i.e., < +1‰). This indicates that even under very intensive manuring, individual pulses may still exhibit low $\delta^{15}\text{N}$ values (< 1‰), making them indistinguishable from unmanured crops. However, one factor which consistently distinguishes the control samples from manured samples is the wide variation in $\delta^{15}\text{N}$ values within a single treatment type/plot.

The results outlined here are consistent with our previous study (Treasure et al. 2016) and support previous research by Fraser et al. (2011) who examined the relationship between manuring and $\delta^{15}\text{N}$ values and pulses through a series of field studies and experiments. Fraser et al. (2011) documented high $\delta^{15}\text{N}$ values for faba/broad bean (+2.2‰ \pm 1.4‰) and Cyprus vetch (> +1.5‰) in a farm study in Evvia, Greece, where cultivation plots were subject to prolonged, intensive manuring (sheep/goat dung) forming an ‘artificial’ dung-soil. Very slightly elevated faba/broad bean $\delta^{15}\text{N}$ values (> +1‰) were recorded in cultivation plots in Asturias, Spain, which were biennially manured, although most $\delta^{15}\text{N}$ values were < +1‰ (Fraser et al. 2011). Manuring rates at Evvia and Asturias were not directly measured since it is difficult to accurately quantify application rates in ‘real’ farming situations (cf. Halstead 2014: 218–225). In Morocco, Bogaard et al. (2018) also recorded low $\delta^{15}\text{N}$ values (< +1‰) for a range of pulse crops despite manuring, although water availability and temperature may have played a role here. Overall, the $\delta^{15}\text{N}$ values (> +1‰) observed in the farm study at Evvia are closely comparable to our results, confirming that ¹⁵N-enrichment in pulses due to manuring will only be caused in cases of *very intensive* application rates, supported by the results of the statistical tests in Table 2.

Pulse $\delta^{15}\text{N}$ values reflect the relative contributions of nitrogen obtained via atmospheric N₂-fixation and uptake of soil nitrogen. Plants assimilating a high proportion of nitrogen via atmospheric N₂-fixation are expected to have $\delta^{15}\text{N}$ values close to 0‰ \pm 1‰; this is seen in the control samples analysed in this study. Manuring may increase pulse $\delta^{15}\text{N}$ values since high soil mineral nitrogen has been documented to suppress atmospheric N₂-fixation, with plants instead becoming more dependent on soil organic nitrogen (e.g., Unkovich and Pate 2000). However, manuring has also been shown to *increase* atmospheric N₂-fixation rates (Jannoura et al. 2014). Despite this, in cases where soil mineral nitrogen is very high and significantly ¹⁵N-enriched due to *very intensive* manuring, pulse $\delta^{15}\text{N}$ values may increase

above the value expected for plant assimilating atmospheric N (i.e., > +1‰).

The magnitude of ¹⁵N-enrichment also partly depends on the $\delta^{15}\text{N}$ value of the manure or ‘bio-fertiliser’ applied. Historical and ethnographic sources indicate that a wide range of amendments were added to soils in the past, including manure from a range of different animal species (e.g., sheep/goats, cattle, pigs, birds), as well as other material including human faeces or ‘nightsoil’ (human faecal waste), domestic refuse, ashes, turves, marl/lime, seaweed, and fish amongst others (Jones 2012). In general, a positive correlation exists between the $\delta^{15}\text{N}$ value of the manure/fertiliser applied and plant $\delta^{15}\text{N}$ values (Szpak 2014). For example, in a previous study at Durham University by Gröcke et al. (2021), ‘Celtic black broad bean’ $\delta^{15}\text{N}$ values ranged between +5.5‰ to +18.1‰ in plots which were amended with fish carcasses of Atlantic cod which had a $\delta^{15}\text{N}$ value of \sim +15.5‰. Similarly, extremely high $\delta^{15}\text{N}$ values (> +15‰) have been recorded in experiments where Common bean (*Phaseolus vulgaris*) was cultivated in plots treated with seabird guano which had a $\delta^{15}\text{N}$ of > +20‰ (Szpak et al. 2014). In comparison, the $\delta^{15}\text{N}$ value of terrestrial herbivore dung/manure (e.g., cattle, sheep/goat) is typically < +10‰ (Szpak 2014). However, it should be noted here that if the domesticated herbivores were consuming enriched ¹⁵N food sources (e.g., cereals amended with seaweed and/or Atlantic cod), this threshold may need revising (see Gröcke et al. 2021), though pulses cultivated in plots manured with herbivore dung from animals not fed on marine resources are therefore unlikely to exhibit $\delta^{15}\text{N}$ values of > 6‰. However, the impact of other soil amendments on crop stable isotope ratios has yet to be systematically examined in detail.

Our results are likely to be broadly applicable to a range of pulse crops, especially those cultivated in temperate areas. However, further research is required to examine the impact of manuring on other major pulse crop species and cultivars. For example, N₂-fixation rates can vary between different species (e.g., peas, lentil, chickpea, faba/broad bean), whilst some faba/broad bean (*V. faba*) cultivars have been demonstrated to maintain high N₂-fixation rates even under conditions of high soil mineral nitrogen (Hardason et al. 1991; Turpin et al. 2002). Equally, we do not currently have a clear understanding of whether other environmental variables (e.g. drought stress, salinity, waterlogged soils) could stimulate anomalously ‘high’ and/or ‘low’ $\delta^{15}\text{N}$ values in pulses, irrespective of whether a crop has been manured.

Implications for reconstructing farming practices and palaeodiets

The rates of manure application analysed here can be characterised as *very intensive* compared to those routinely

recorded in ethnographic studies (Bogaard 2012; Halstead 2014). This would only be feasible in small, cultivated plots or gardens, probably close to settlements and animal pens due to the difficulty of transporting sufficient manure long distances (Jones 2005). For example, in high intensity cultivation regimes in Morocco with hand tilling, weeding, irrigation and manuring, Bogaard et al. (2018) observed manuring rates of approximately 100 t/ha (10 kg/m²) in very small arable fields (0.01 ha). Intensive cultivation regimes were probably more widespread prior to the modern period and many farming regimes would have encompassed elements of both extensive and intensive cultivation practices (Halstead 1987). In pre-mechanised agriculture, cereal cultivation has often (though not invariably) been undertaken on an extensive scale, whereas intensively cultivated and manured gardens near settlements have focused on labour demanding crops such as pulses and vegetables (Hillman 1973; Halstead 1987). Pulses, such as faba/broad beans, were also grown in rotation with other crops due to their nitrogen-fixing capabilities, with the harvested plants ploughed back into the soil, releasing the nitrogen as the plants decomposed.

From a palaeodietary perspective, pulses cultivated intensively in gardens may not have $\delta^{15}\text{N}$ values around 0‰ (e.g., DeNiro and Epstein 1981; van Klinken et al. 2002), emphasising the importance of directly measuring archaeobotanical crops for $\delta^{15}\text{N}$ to develop baselines for dietary reconstructions (e.g., Fraser et al. 2013; Gröcke et al. 2021). In some cases, there may not always have been a clear separation in $\delta^{15}\text{N}$ values between non-N₂ fixers (e.g., cereals) and N₂-fixers such as a pulses, particularly where the manure and/or bio-fertiliser applied is ¹⁵N-enriched (cf. Warinner et al. 2013; Szpak et al. 2013; Gröcke et al. 2021). The leaves and stems of the amended pulse crops could also have been fed to domestic animals as fodder, subsequently having the potential to raise the herbivore $\delta^{15}\text{N}$ baseline (i.e., Gröcke et al. 2021).

Crop $\delta^{13}\text{C}$ values can be used alongside $\delta^{15}\text{N}$ values to investigate cultivation conditions and husbandry practices (i.e., Ferrio et al. 2020). Our results indicate that manuring generally had a small and inconsistent effect on $\delta^{13}\text{C}$ values in Celtic bean, although there may be large variation in $\delta^{13}\text{C}$ values (> 3‰) within a single crop cultivated under the same/similar conditions. Consequently, it may not be possible to infer whether an archaeobotanical pulse assemblage contains crops cultivated in a single field, or alternatively if crops were sourced from a wider landscape area from multiple fields, though further experimental research is needed to analyse this (cf. Aguilera et al. 2018; Diffey et al. 2020; Hamerow et al. 2020).

Application to archaeobotanical assemblages

The application of this isotopic approach to archaeobotanical assemblages requires careful sample selection since it is of central importance that ¹⁵N-enrichment can be ruled out as deriving from either charring and/or contamination (Braadbaart et al. 2004; Vaiglova et al. 2014; Nitsch et al. 2015; Brinkkemper et al. 2018; Szpak and Chiou 2020; Hartman et al. 2020). Current research indicates that reliable stable isotope values can be obtained from pulses charred within the ‘optimal charring window’, i.e., low-temperatures between 220 and 300 °C (Braadbaart et al. 2004; Nitsch et al. 2015; Stroud et al. 2023). Pulse seeds charred under these conditions will be in excellent condition and exhibit minimal distortion. Some archaeobotanical assemblages will not be sufficiently preserved to meet this selection criteria, and thus it is recommended that the preservation of individual specimens is recorded prior to isotope analysis. Single-entity sample analysis is also recommended, as opposed to bulk analysis of many seeds, since the variability in $\delta^{15}\text{N}$ (and d^{13}C) is an important factor in the identification of manuring practices and/or environmental conditions. Provided that the above criteria are met, we suggest that stable isotope analysis is a powerful method for investigating past crop husbandry practices for pulse crops.

We furthermore suggest that $\delta^{15}\text{N}$ values of > +1.5‰ obtained on archaeobotanical faba/broad bean samples most likely reflect very intensive manuring. In particular, beans cultivated on manured soils are likely to be characterised by wide variation in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, including outlier samples with anomalously high $\delta^{15}\text{N}$ values (> +5‰).

Conclusion

Three main conclusions can be taken from our study. Firstly, manuring at both intensive (70–80 t/ha) and very intensive (150–160 t/ha) rates increased biomass production, seed number and seed length. Secondly, manuring did not cause consistent variation or offset in $\delta^{13}\text{C}$ values, though the variation in $\delta^{13}\text{C}$ within plots and within treatment types can be significant (> 3‰). Finally, only very intensive manuring (150–160 t/ha) results in consistently elevated pulse $\delta^{15}\text{N}$ values (> +1.5‰), with potential ¹⁵N-enrichment of up to +5‰. However, even under very intensive manuring, individual pulse seeds may still exhibit low $\delta^{15}\text{N}$ values (< 1‰), making them indistinguishable from unmanured crops. It is therefore recommended that each sample submitted for stable isotope analysis is single-entity (e.g. from one identified pulse), so that the isotopic variability of the archaeobotanical assemblage from the stratigraphic context can be adequately assessed and interpreted.

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Author contributions ET, MC and DG designed and conducted the experiment. RB and MC built and maintained the experimental plot layout. ET, MC, RB and JL conducted the biomass measurements. DG and ET conducted the stable isotope analyses. ET and MC wrote the manuscript, with contributions from DG, RB, SJ and JL.

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Declarations

Competing interests The authors declare no competing interests.

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