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ORIGINAL PAPER

The influence of manuring on stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) in Celtic bean (*Vicia faba* L.): archaeobotanical and palaeodietary implications

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Abstract This paper examines the impact of animal manure on $\delta^{15}N$ and $\delta^{13}C$ values in a legume, Celtic Black broad bean (*Vicia faba*). In a field experiment, *V. faba* was cultivated in plots treated with farmyard manure and pure sheep manure. The results indicate that highly intensive manuring can increase $\delta^{15}N$ values in beans, stems, leaves and pods. In comparison, manuring had a relatively small impact on $\delta^{13}C$ values. In terms of palaeodietary reconstructions, the high $\delta^{15}N$ values in very intensively manured beans (+3‰) are equivalent to the trophic-level effect. Based on the experimental results, it is suggested that high $\delta^{15}N$ values in archaeobotanical remains of *V. faba* may be attributable to small-scale cultivation with intensive manuring.

Keywords Stable isotopes · Nitrogen · Carbon · Manuring · Legume · Palaeodiet

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Introduction

Stable isotope analysis is becoming increasingly widespread in archaeobotanical research as a powerful method for investigating agricultural practices and land use patterns (Aguilera et al. 2008; Bogaard et al. 2013; Fraser et al. 2013; Heaton et al. 2009; Kanstrup et al. 2014; Masi et al. 2014; Vaiglova et al. 2014). Plant stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios vary in relation to a range of environmental factors (Dawson et al. 2002) including agricultural practices, and these variations can be analysed in archaeobotanical remains (Fiorentino et al. 2015; Szpak 2014). In particular, recent research has been directed towards the identification of manuring in cereals and pulses (Bogaard et al. 2007, 2013; Fraser et al. 2011; Kanstrup et al. 2011, 2014). Since manuring is closely linked to intensive land use patterns, it is an important area of research into past crop husbandry regimes (Bogaard 2012).

Field experiments and farm studies indicate that animal manuring increases cereal $\delta^{15}N$ values (≥6‰) (Bogaard et al. 2007; Bol et al. 2005; Fraser et al. 2011; Kanstrup et al. 2011). In comparison, manuring has minimal effect on $\delta^{15}N$ values in legumes, except where very intensively applied over a long duration (Fraser et al. 2011; Styring et al. 2014). There is a requirement to further examine the relationship between manuring intensity and legume $\delta^{15}N$ values (Styring et al. 2014). In terms of palaeodietary reconstructions, it is important to assess the impact of manuring on legume $\delta^{15}N$ values as a source of ^{15}N enrichment in animal and human tissues (Bogaard et al. 2007; Fraser et al. 2011; Hedges and Reynard 2007).

Plant $\delta^{13}C$ values have been primarily applied to investigate crop water management practices (Fiorentino et al. 2015). However, other environmental factors that alter $\delta^{13}C$ need to be considered (Stokes et al. 2011). The relationship between plant $\delta^{13}C$ values and manuring is not clearly understood with both positive

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and negative shifts observed in $\delta^{13}C$ (Kanstrup et al. 2011; Senbayram et al. 2008; Szpak et al. 2012b; Wallace et al. 2013).

The aim of this study is to analyse the influence of manuring on $\delta^{13}C$ and $\delta^{15}N$ values in the legume, Celtic Black broad bean (*Vicia faba* L.). *V. faba* is frequently recovered in archaeobotanical assemblages across the Near East and Europe from the Neolithic and Bronze Age onwards (see Colledge and Conolly 2007; Stika and Heiss 2013). A recent study identified extensive evidence for *V. faba* in Britain in the Neolithic-Iron Age, totalling over 70 archaeobotanical assemblages containing Celtic Bean, and suggested that, in some areas, it may have been cultivated on a small 'garden' scale in intensively managed plots treated with animal manure (Treasure 2014). In order to accurately assess the relationship between $\delta^{13}C$ and $\delta^{15}N$ values in *V. faba* and manuring, it is necessary to undertake field experiments where the rates of manure application can be quantified.

Methodological background

Nitrogen isotopes ($\delta^{15}N$)

Plant $\delta^{15}N$ values reflect the net effect of a range of factors, including the form of nitrogen acquired (NH_4^+, NO_3^-, N_2) and the method of nitrogen assimilation (uptake of soil nitrogen, fixation of atmospheric N_2) (Evans 2001; Högberg 1997).

Pulses are harvested from leguminous plants which can assimilate nitrogen through fixation of atmospheric N_2 via symbiotic bacteria (rhizobia) in the roots (Franche et al. 2009; Howard and Rees 1996). As N_2 fixation involves minimal fractionation, legumes dependent on fixation as a source of nitrogen have $\delta^{15}N$ values typically around 0‰, reflecting atmospheric N_2 (i.e., $\delta^{15}N_{air}$=0‰) (Kohl and Shearer 1980; Shearer and Kohl 1986; Virginia and Delwiche 1982). A range of factors, however, can influence N_2 fixation, in particular, soil nitrogen availability (Liu et al. 2011). In soil N-rich environments, N_2 fixation is inhibited and legumes preferentially take up nitrogen from the soil (Ledgard et al. 1996; Peoples et al. 2009; Vinther 1998). The nitrogen isotopic composition of legumes taking up nitrogen from soil (rather than N_2 fixation) will reflect the $\delta^{15}N$ value of the soil nitrogen (Andrews et al. 2011). As manuring increases soil mineral nitrogen and $\delta^{15}N$ values (Choi et al. 2003; Simpson et al. 1999; Watzka et al. 2006), it has the potential to increase $\delta^{15}N$ values in legumes above 0‰.

In cereals (non-N_2-fixing plants), animal manure significantly increases $\delta^{15}N$ values (up to +10‰) due to the uptake of ^{15}N-enriched soil (Bogaard et al. 2007; Bol et al. 2005; Fraser et al. 2011; Kanstrup et al. 2011, 2012). In comparison, a recent study suggests that only very intensive animal manure application, in excess of >20–35 t/ha, alters $\delta^{15}N$ in *V. faba* due to the preferential fixation of atmospheric N_2 (Fraser et al. 2011; Styring et al. 2014). The largest increase in *V. faba* $\delta^{15}N$ values (+2.2±1.4‰) was observed in a farm study in Evvia, Greece, where very intensive manuring creates artificial 'dung-soil' (Fraser et al. 2011). However, the rate of manure application (t/ha) was not measured in the Evvia farm study.

Carbon isotopes ($\delta^{13}C$)

During photosynthesis, C_3 plants (cereals, legumes) discriminate against ^{13}C due to the preferential use of ^{12}C by the enzyme RuBisCO during carbon fixation (Lloyd and Farquhar 1994; O'Leary 1981). The amount of ^{13}C discrimination is closely linked with stomatal conductance (intrinsic water use efficiency) (Farquhar and Sharkey 1982; Farquhar et al. 1989; O'Leary 1988). Restricted water availability increases stomatal closure and reduces ^{13}C discrimination (Chaves 1991; Farquhar et al. 1989). In comparison, during high water availability, stomata are open, increasing ^{13}C discrimination (Chaves 1991; Farquhar et al. 1989). Studies have identified a causal link between $\delta^{13}C$ values in plants and water availability in greenhouse and farm studies (Araus et al. 1997; Farquhar and Richards 1984; Flohr et al. 2011; Wallace et al. 2013).

However, a wide range of other environmental factors can alter $\delta^{13}C$ values in plants including salinity, light intensity, temperature and nitrogen availability (Condon et al. 1992; Gröcke 1998, 2002; Heaton 1999; O'Leary 1981; Tieszen 1991). The relationship between plant $\delta^{13}C$ values and manuring is complex with both positive and negative relationships observed (Kanstrup et al. 2011; Senbayram et al. 2008; Szpak et al. 2012b; Wallace et al. 2013). At present, there is no published data which specifically focuses on the impact of manuring on $\delta^{13}C$ values in *V. faba*.

Materials and methods

Experimental design

Celtic Black broad beans (*V. faba* L.) were cultivated in three 1-m^2 outdoor plots at Durham University Botanic Gardens between May and September 2013. The Celtic Black broad beans used are a heritage variety which produces small-rounded seeds which are morphologically similar to prehistoric finds of *V. faba*. One plot acted as a control, and two plots were treated with manure and decomposed leaf litter (Table 1). All the available plants were harvested and the plant height, number of ripe/un-ripe pods, number of beans and dimensions of each bean were recorded.

One plant from each plot was randomly selected for isotopic analysis and air-dried. Samples of bean cotyledons, bean testae, pods, leaves and stem were analysed. All of the pods and beans available for each selected plant were analysed. The cotyledon was sampled separately, as the testa is rarely preserved in archaeobotanical remains of *V. faba*. The position of

Table 1 Field treatments for the plots used in this study

		Treatment (t/ha)	Detail
Plot 1	Control	n/a	No amendment.
Plot 2	Amended	70	14 l of farmyard manure (animal manure/straw) with 14 l of decomposed leaf litter mixed into the soil
Plot 3	Midden	100	20 l of pure sheep manure (approx.) with 20 l of decomposed leaf litter not mixed into the soil

each pod on the plant and each bean within individual pods was recorded. This detailed sampling methodology enables analysis of within-plant $\delta^{13}C$ and $\delta^{15}N$ variation. Dried soil and manure samples were sieved at <1 mm. All samples were homogenised in an agate pestle and mortar.

Stable isotope analysis

Stable isotope measurements were performed at Durham University using a Costech Elemental Analyser (ECS 4010) coupled to a Thermo Finnigan Delta V Advantage isotope mass spectrometer. Carbon isotope ratios are Craig-corrected for ^{17}O contribution and reported in standard delta (δ) notation in per mil (‰) relative to VPDB. Nitrogen isotope ratios are reported relative to AIR. Data accuracy is monitored through routine analyses of in-house standards, which are stringently calibrated against international standards (e.g., USGS 40, USGS 24, IAEA 600, IAEA N1, IAEA N2). Analytical uncertainty for $\delta^{13}C$ and $\delta^{15}N$ measurements is typically ±0.1‰ for replicate analyses of the international standards and typically <0.2‰ on replicate sample analysis. Total organic carbon and nitrogen data was obtained as part of the isotopic analysis using an internal standard (i.e., glutamic acid, 40.82 % C and 9.52 % N).

Results

Biomass analysis

The results of the biomass analysis are presented in Table 2. Six plants from the midden plot suffered insect damage and did not produce any pods. Plants in the amended plot were taller than in the control plot. In comparison, the heights of plants in the control and midden plots are identical (though this may be due to insect damage in the midden plants). Pod and bean yield was highest in plants in the amended plot. The comparative results for the midden plot are significantly lower, though, as noted above, this may be due to insect damage. Bean dimensions increased (particularly length) in the amended and midden plots.

Stable isotope analyses

Mean $\delta^{13}C$ and $\delta^{15}N$ values for the samples analysed are presented in Table 3. Figure 1 presents the $\delta^{13}C$ and $\delta^{15}N$ values for all the plant and soil samples analysed. The supplementary data includes the results for each sample in addition to C/N atomic ratio, %C and %N results.

Soil and manure analyses

Mean soil $\delta^{15}N$ values in the amended (5.5±0.4‰) and midden (8.1±1.7‰) plots are significantly higher than in the control plot (4.6±0.2‰). Mean $\delta^{15}N$ values for the farmyard manure were 7.7±0.3‰ and, for the sheep manure, 7.5±0.2 ‰. In the amended and midden plots, mean $\delta^{13}C$ values are lower than in the control plot.

Plant analyses

Mean $\delta^{15}N$ values in the control samples varied between −1 and 0.7‰. Mean $\delta^{15}N$ values in cotyledons were 1.5±0.2‰ in the amended samples and 2.6±0.3‰ midden samples. Pods, stems and leaves were ^{15}N enriched in the amended and midden samples. There is small variation in $\delta^{15}N$ values between cotyledons sampled from the same pod and plant. $\delta^{15}N$ variation between leaves, stems and pods was typically ≤1‰, with the largest offset (1.7‰) between the midden pod and midden stem samples (see Electronic supplementary material). Testa $\delta^{15}N$ values are significantly lower than cotyledon $\delta^{15}N$ values. Manuring intensity and $\delta^{15}N$ values are positively correlated.

Mean $\delta^{13}C$ values in the amended and midden samples are similar to the control samples, with small increases observed in some samples of up to 1.7‰. Within-plant $\delta^{13}C$ variation between leaves, stems and pods was minimal. There is only a small variation in $\delta^{13}C$ values between cotyledons sampled from the same pod and plant.

Discussion

Soil and manure $\delta^{13}C$ and $\delta^{15}N$ variability

In agreement with previous studies, manuring increased soil $\delta^{15}N$ values (Bol et al. 2005; Kanstrup et al. 2011; Senbayram et al. 2008; Watzka et al. 2006). The mean $\delta^{15}N$ values for the farmyard manure (7.7±0.3‰) and sheep manure (7.5±0.2‰) are high for animal manure (cf. Szpak 2014). Mean $\delta^{13}C$ values were lower in amended and midden plots as animal

Table 2 Biomass analysis of the Celtic Black broad beans from this study

		Control	Amended	Midden
Number of plants		19	20	19
Height (cm)		45±10	56±9**	45±10
Number of pods		33	40	21
Number of beans		101	129	56
Dimensions of beans (mm)	Width	6.64±0.86	6.81±0.72	6.79±0.79
	Length	9.04±1.26	9.54±1.02**	10.05±1.18***
	Depth	7.53±1.14	7.79±0.87	8.14±0.90**

Plant height and dimensions of beans were statistically compared between the control plot and amended plot, and the control plot and midden plot using an unpaired samples *t* test. ± is one standard deviation

$^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$

manure is ^{13}C-depleted (Bol et al. 2005). Previous studies have observed decreases in δ^{13}C values in manured soils (Gerzabek et al. 1997; Senbayram et al. 2008).

Plant δ^{13}C variability

Plant δ^{13}C values were minimally affected by manuring, displaying only small increases, typically around +1‰. These results are consistent with previous studies which indicate that manuring may be a source of small variation in ^{13}C values (Senbayram et al. 2008; Szpak et al. 2012b; Wallace et al. 2013). Small increases in δ^{13}C values in response to nitrogen fertilisation observed in previous studies may be related to increased plant biomass which can limit stomatal conductance causing less ^{13}C discrimination (Jenkinson et al. 1995; Serret et al. 2008).

Plant δ^{15}N variability

The results presented in this study indicate that very intensive animal manuring (>70 t/ha) can increase δ^{15}N values (+3‰) in *V. faba*. There is little δ^{15}N variation between plant tissues,

Table 3 δ^{15}N and δ^{13}C analysis of the Celtic Black broad beans from this study

Tissue	Plot	Number of samples	δ^{13}C	δ^{15}N
Cotyledon	Control	7	−30.2±0.1	0.7±0.1
	Amended	7	−29.2±0.2***	1.5±0.2***
	Midden	3	−28.8±0.3***	2.6±0.3***
Testa	Control	7	−32.1±0.3	−1.0±0.5
	Amended	7	−31.3±0.2***	−1.0±0.4
	Midden	3	−31.1±0.1***	0.9±0.3***
Pod	Control	2	−31.9±0.5	0.2±0.1
	Amended	2	−31.9±0.0	1.4±0.0**
	Midden	1	−31.3	1.7
Leaf	Control	2	−31.6±0.2	−0.1±0.1
	Amended	2	−31.6±0.0	1.1±0.1**
	Midden	1	−31.2	3.0
Stem	Control	2	−31.0±0.4	−0.3±0.1
	Amended	1	−30.9	0.7
	Midden	3	−29.3±0.3*	3.0±0.7**
Soil	Control	4	−25.3±0.1	4.6±0.2
	Amended	4	−26.7±0.5**	5.5±0.4**
	Midden	4	−29.9±1.3***	8.1±1.7**
Farmyard manure	Amended	2	−29.3±0.2	7.7±0.3
Sheep manure	Midden	2	−30.0±0.2	7.5±0.2

Results were statistically compared (excluding the manure samples) between the control plot and amended plot, and the control plot and midden plot using an unpaired samples *t* test. ± is one standard deviation

$^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$

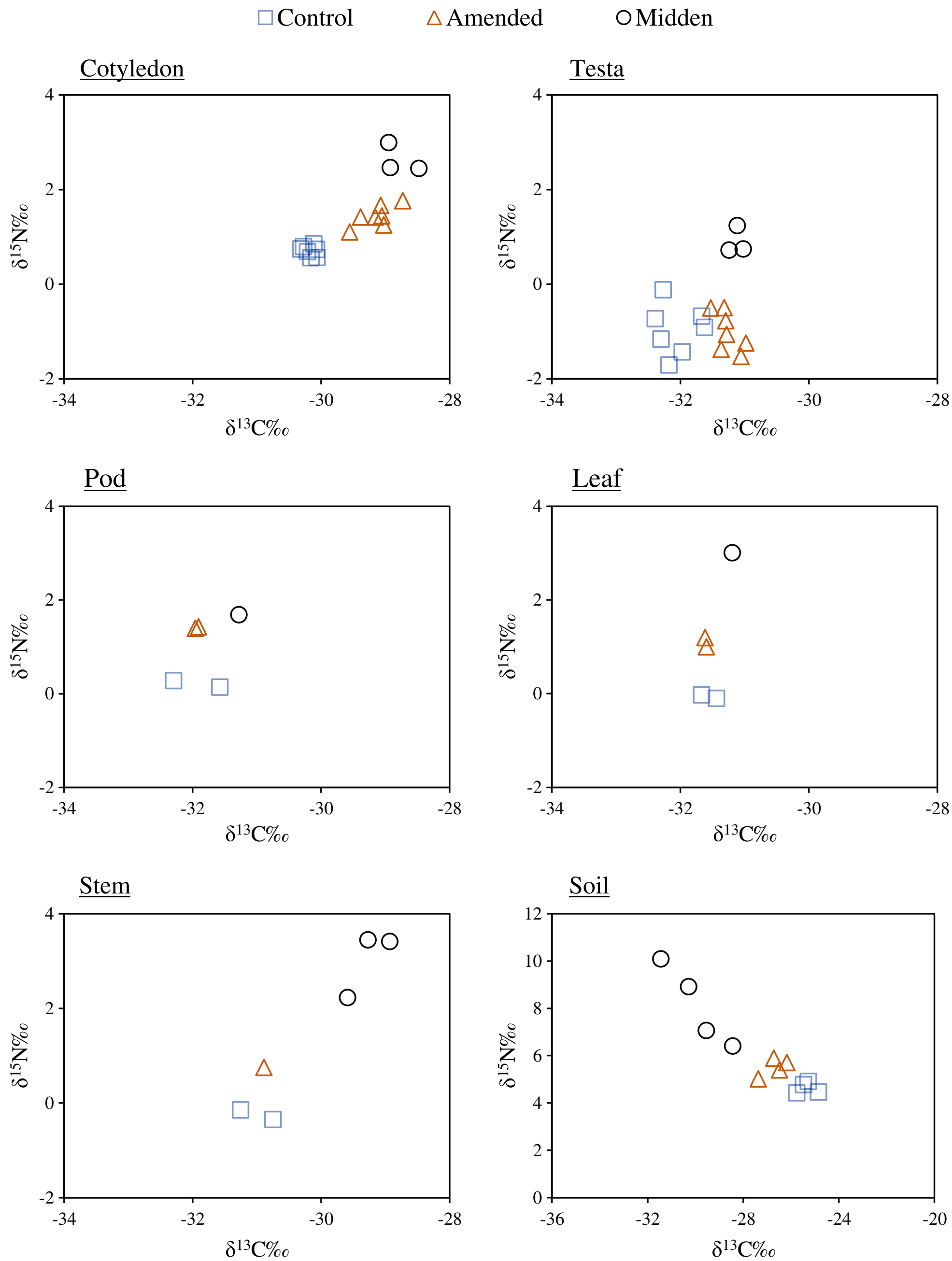

Fig. 1 $\delta^{15}N$ and $\delta^{13}C$ values for each sample (excluding the manure samples). Note the different scale on the soil graph

with the exception of testa samples, comparable with results from previous studies (López-Bellido et al. 2010; Nebiyu et al. 2014). Control sample $\delta^{15}N$ values varied between 0±1‰ and are consistent with fixation of atmospheric N_2 in *V. faba* (Fan et al. 2006; López-Bellido et al. 2011; Nebiyu et al. 2014; Tryderman et al. 2004; Unkovich 2013). In contrast, the elevated $\delta^{15}N$ values in the amended and midden samples indicate preferential uptake of soil mineral nitrogen in comparison to atmospheric N_2 fixation. The $\delta^{15}N$ values in the midden plot are higher than in the amended plot due to the application of pure manure compared to farmyard manure (i.e., a mixture of straw and manure).

The results presented here agree with a previous study which indicated that only very intensive manuring can significantly alter $\delta^{15}N$ values in *V. faba* due to the preferential fixation of atmospheric N_2 (Fraser et al. 2011; Styring et al. 2014). Fraser et al. (2011) only observed a large increase in $\delta^{15}N$ values for *V. faba* in a farm study at Evvia, Greece, where very intensive manuring (sheep/goat dung) creates an artificial dung-soil. The quantity of manure applied (t/ha) in the Evvia farm study could not be measured, though the soil conditions appear to be similar to the midden plot in this study.

The relationship between N_2 fixation and animal manuring in legumes is an area which requires further research. It is not clear why $\delta^{15}N$ values in legumes are only altered by very intensive manuring as it requires less energy to take up soil mineral nitrogen compared to fixing atmospheric N_2 (Andrews et al. 2009). A possible explanation may be due to ability of certain varieties of *V. faba* to continue atmospheric N_2 fixation in the presence of high soil mineral nitrogen (Köpke and Nemecek 2010). Recently, Szpak et al. (2014) observed large ^{15}N enrichment (+16‰) in a legume (common garden bean, *Phaseolus vulgaris* L.) amended with seabird guano which has a very high $\delta^{15}N$ value (> +20‰) compared with animal manure (Szpak et al. 2012a; Szpak 2014). This indicates that manures which are high in plant available nitrogen and have high $\delta^{15}N$ values can significantly enrich ^{15}N in legumes and suppress N_2 fixation compared to animal manure (Szpak et al. 2014). In comparison, a recent study demonstrated that animal manure increased N_2 fixation in peas (*Pisum sativum* L.; Jannoura et al. 2014).

Archaeological implications

The results of this study suggest that *intensive* manuring of *V. faba* may be identifiable in archaeobotanical remains using nitrogen isotope analysis (cf. Fraser et al. 2011). The high intensity of manuring required to effect the nitrogen isotopic composition of pulses indicates that pulse $\delta^{15}N$ values can reflect the scale of cultivation. In recent farming contexts, intensity of manuring is closely correlated with the scale of cultivation, with smaller plots receiving intensive manure application (Bogaard et al. 2000; Jones 2005). In Evvia, Greece, *V. faba* is cultivated in small infield (garden) areas, some of which are very intensively manured, creating an artificial dung-soil (Jones et al. 1999). Similarly, in Asturias, Spain, *V. faba* is cultivated in small intensively manured plots that are rotated with cereals (Charles et al. 2002). It is suggested that high $\delta^{15}N$ values in archaeobotanical remains of *V. faba* may indicate small-scale cultivation with very intensive manuring. The results of this study should be viewed as preliminary in character, and further research is currently ongoing to explore $\delta^{13}C$ and $\delta^{15}N$ variability between plants cultivated in the same plot. For example, Wallace et al. (2013) have demonstrated large variation in $\delta^{13}C$ values in *V. faba* cultivated under similar conditions. Variation in plant isotope values is expected in traditional farming regimes where growing conditions can be variable (Wallace et al. 2013)

In terms of palaeodietary reconstructions, measurement of plant $\delta^{15}N$ values is necessary in order to accurately reconstruct baseline data and interpret $\delta^{15}N$ values in animal and human tissues (Casey and Post 2011; Hedges and Reynard 2007). In particular, Fraser et al. (2013) have demonstrated that the plant component of diets can be assessed with greater accuracy through direct measurement of archaeological plant $\delta^{15}N$ values. The impact of manuring on cereal $\delta^{15}N$ values (up to 10‰) is significantly higher than the Celtic Black broad beans analysed in this study. Despite this, the enrichment in $\delta^{15}N$ between the control and the midden samples (~3‰) is equivalent to the trophic-level effect (3–5‰, Bocherens and Drucker 2003) and, hence, could subsequently cause ^{15}N enrichment in animal and human tissues. This is particularly significant as palaeodietary studies typically consider legumes to have $\delta^{15}N$ values around 0‰ (DeNiro and Epstein 1981; van Klinken et al. 2002).

In manured cereals, there is a large offset in $\delta^{15}N$ values between the grain and chaff (2.4±0.8‰), suggesting that the use of chaff for animal fodder and grain for human consumption will result in significantly different nitrogen isotopic signatures between animals and humans (Bogaard et al. 2007; Fraser et al. 2011). In comparison, the results of this study indicate a comparatively small offset in $\delta^{15}N$ values of manured *V. faba* between different plant components. This is significant as ethnographic evidence indicates that *V. faba* was used as a source of fodder, either the seeds, chaff or whole plants (Forbes 1996; Halstead 2014).

Conclusion

The results of this study indicate that highly intensive animal manuring can increase $\delta^{15}N$ values in legumes. Celtic Black broad beans (*V. faba*) displayed significantly higher $\delta^{15}N$ values in intensively manured plots. In comparison, manure minimally affected $\delta^{13}C$ values, indicating that manuring may only be a source of small variation in $\delta^{13}C$ values. In terms of palaeodietary reconstructions, manuring increased $\delta^{15}N$ values on a scale equivalent to a single step in trophic level. Based on the experimental results presented here, it is suggested that high $\delta^{15}N$ values in archaeobotanical remains of *V. faba* should be attributed to small-scale cultivation with very intensive manuring.

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References

Aguilera M, Araus JL, Voltas J, Rodríguez-Ariza MO, Molina F, Rovira N, Buxó R, Ferrio JP (2008) Stable carbon and nitrogen isotopes and quality traits of fossil cereal grains provide clues on sustainability at the beginnings of Mediterranean agriculture. Rapid Commun Mass Spectrom 22:1653–1663

Andrews M, Lea PJ, Raven JA, Azevedo RA (2009) Nitrogen use efficiency. 3. Nitrogen fixation: genes and costs. Ann Appl Bot 155:1–13

Andrews M, James EK, Sprent JI, Boddey RM, Gross E, Bueno dos Reis F (2011) Nitrogen fixation in legumes and actinorhizal plants in natural ecosystems: values obtained used ^{15}N natural abundance. Plant Ecolog Divers 4:131–140

Araus JL, Febrero A, Buxó R, Rodríguez-Ariza MO, Molina F, Camalich MD, Martín D, Voltas J (1997) Identification of ancient irrigation practices based on the carbon isotope discrimination of plant seeds: a case study from the south-east Iberian Peninsula. J Archaeol Sci 24: 729–740

Bocherens H, Drucker D (2003) Trophic level isotopic enrichment of carbon and nitrogen in bone collagen: case studies from recent and ancient terrestrial ecosystems. Int J Osteoarchaeol 13:46–53

Bogaard A (2012) Middening and manuring in Neolithic Europe: issues of plausibility, intensity and archaeological method. In: Jones RL (ed) Manure matters: historical, archaeological and ethnographic perspectives. Ashgate, Farnham, pp 25–39

Bogaard A, Charles M, Haltead P, Jones G (2000) The scale and intensity of cultivation: evidence from weed ecology. In: Halstead P, Fredrick C (eds) Landscape and land use in postglacial Greece. Sheffield Academic Press, Sheffield, pp 129–134

Bogaard A, Heaton THE, Poulton P, Merbach I (2007) The impact of manuring on nitrogen isotope ratios in cereals: archaeobotanical implications for reconstruction of diet and crop management practices. J Archaeol Sci 34:335–343

Bogaard A, Fraser R, Heaton THE, Wallace M, Vaiglova P, Charles M, Jones G, Evershed RP, Styring AK, Andersen NH, Arbogast R-M, Bartosiewicz L, Gardeisen A, Kanstrup M, Maier U, Marinova E, Ninov L, Schäfer M, Stephan E (2013) Crop manuring and intensive land management by Europe's first farmers. Proc Natl Acad Sci 110: 12589–12594

Bol R, Eriksen J, Smith P, Garnett MH, Coleman K, Christensen BT (2005) The natural abundance of ^{13}C, ^{15}N, ^{34}S, and ^{14}C in archived (1923-2000) plant and soil samples from the Askov long-term experiments on animal manure and mineral fertilizer. Rapid Commun Mass Spectrom 19:3216–3226

Casey MM, Post DM (2011) The problem of isotopic baseline: reconstructing the diet and trophic position of fossil animals. Earth Sci Rev 106:131–148

Charles M, Bogaard A, Jones G, Hodgson J, Halstead P (2002) Towards the archaeobotanical identification of intensive cereal cultivation: present-day ecological investigation in the mountains of Asturias, northwest Spain. Veg Hist Archaeobot 11:133–142

Chaves MM (1991) Effects of water deficits on carbon assimilation. J Exp Bot 42:1–16

Choi W-J, Ro H-M, Hobbie EA (2003) Patterns of natural ^{15}N in soils and plants from chemically and organically fertilized uplands. Soil Biol Biochem 35:1493–1500

Colledge S, Conolly J (eds) (2007) The origins and spread of domestic plants in Southwest Asia. Left Coast Press, Walnut Creek

Condon AG, Richards RA, Farquhar GD (1992) The effect of variation in soil water availability, vapour pressure deficit and nitrogen nutrition on carbon isotope discrimination in wheat. Aust J Agric Res 43: 935–948

Dawson TE, Mambelli S, Plamboeck AH, Templer PH, Tu KP (2002) Stable isotopes in plant ecology. Annu Rev Ecol Syst 33:507–559

DeNiro MJ, Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals. Geochim Cosmochim Acta 45:341–351

Evans RD (2001) Physiological mechanisms influencing plant nitrogen isotope composition. Trends Plant Sci 6:121–126

Fan F, Zhang G, Song Y, Sun J, Bao X, Guo T, Li L (2006) Nitrogen fixation of faba bean (*Vicia faba* L.) interacting with a non-legume in two contrasting intercropping systems. Plant Soil 283:275–286

Farquhar GD, Richards RA (1984) Isotope composition of plant carbon correlates with water-use efficiency of wheat genotypes. Aust J Plant Physiol 11:539–552

Farquhar GD, Sharkey TD (1982) Stomatal conductance and photosynthesis. Annu Rev Plant Physiol 33:317–345

Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon isotope discrimination and photosynthesis. Annu Rev Plant Physiol Plant Mol Biol 40:503–537

Fiorentino G, Ferrio JP, Bogaard A, Araus JL, Riehl S (2015) Stable isotopes in archaeobotanical research. Veg Hist Archaeobot 24: 215–227

Flohr P, Müldner G, Jenkins E (2011) Carbon stable isotope analysis of cereal remains as a way to reconstruct water availability: preliminary results. Water Hist 3:121–144

Forbes H (1996) European agriculture viewed bottom-side upwards: fodder- and forage-provision in a traditional Greek community. Environ Archaeol 1:19–34

Franche C, Lindströ K, Elmerich C (2009) Nitrogen-fixing bacteria associated with leguminous and non-leguminous plants. Plant Soil 125:1–15

Fraser RA, Bogaard A, Heaton T, Charles M, Jones G, Christensen BT, Halstead P, Merbach I, Poulton PR, Sparkes D, Styring AK (2011) Manuring and stable nitrogen isotope ratios in cereals and pulses: towards a new archaeobotanical approach to the inference of land use and dietary patterns. J Archaeol Sci 38:2790–2804

Fraser RA, Bogaard A, Schäfer M, Arbogast R, Heaton THE (2013) Integrating botanical, faunal and human stable carbon and nitrogen isotope values to reconstruct land use and palaeodiet at LBK Vaihingen van der Enz. Baden Württemb World Archaeol 45: 492–517

Gerzabek MH, Pichlmayer F, Kirchmann H, Haberhauer G (1997) The response of soil organic matter to manure amendments in a long-term experiment at Ultuna, Sweden. Eur J Soil Sci 48:273–282

Gröcke DR (1998) Carbon-isotope analyses of fossil plants as a chemostratigraphic and palaeoenvironmental tool. Lethaia 31:1–13

Gröcke DR (2002) The carbon isotope composition of ancient CO_2 based on higher-plant organic matter. Philos Trans R Soc Lond Ser A 360:633–658

Halstead P (2014) Two oxen ahead: pre-mechanized farming in the Mediterranean. Wiley Blackwell, Oxford

Heaton THE (1999) Spatial, species, and temporal variations in the $^{13}C/^{12}C$ ratios of C_3 plants: implication for palaeodiet studies. J Archaeol Sci 26:637–649

Heaton THE, Jones HP, Tsipropoulus T (2009) Variations in the $^{13}C/^{12}C$ ratios of modern wheat grain, and implications for interpreting data from Bronze Age Assiros Toumba, Greece. J Archaeol Sci 36:2224–2233

Hedges REM, Reynard LM (2007) Nitrogen isotopes and trophic level of humans in archaeology. J Archaeol Sci 34:1240–1251

Högberg P (1997) ^{15}N natural abundance in soil-plant systems. New Phytol 137:179–203

Howard JB, Rees DC (1996) Structural basis of biological nitrogen fixation. Chem Rev 96:2965–2982

Jannoura R, Joergensen R, Bruns C (2014) Organic fertilizer effects on growth, crop yield, and soil microbial biomass indices in sole and intercropped peas and oats under organic farming conditions. Eur J Agron 52:259–270

Jenkinson DS, Coleman K, Harkness DD (1995) The influence of fertilizer nitrogen and season on the carbon-13 abundance of wheat straw. Plant Soil 171:365–367

Jones G (2005) Garden cultivation of staple crops and its implications for settlement location and continuity. World Archaeol 37:164–176

Jones G, Bogaard A, Halstead P, Charles M, Smith H (1999) Identifying the intensity of crop husbandry practices on the basis of weed floras. Annu Br Sch Athens 94:167–189

Kanstrup M, Thomsen IK, Andersen AJ, Bogaard A, Christensen BT (2011) Abundance of ^{13}C and ^{15}N in emmer, spelt and naked barley grown on differently manured soils: towards a method for identifying past manuring practice. Rapid Commun Mass Spectrom 25:2879–2887

Kanstrup M, Thomsen IK, Mikkelsen PH, Christensen BT (2012) Impact of charring on cereal grain characteristics: linking prehistoric manuring practice to $\delta^{15}N$ signatures in archaeobotanical material. J Archaeol Sci 39:25–40

Kanstrup M, Holst MK, Jensen PM, Thomsen IL, Christensen BT (2014) Searching for long-term trend in prehistoric manuring practice. $\delta^{15}N$ analyses of charred cereal grains from the 4th to the 1st millennium BC. J Archaeol Sci 51:115–125

Kohl DH, Shearer G (1980) Isotopic fractionation associated with symbiotic N_2 fixation and uptake of NO^{-3} by plants. Plant Physiol 66:51–56

Köpke U, Nemecek T (2010) Ecological services of faba bean. Field Crop Res 115:217–238

Ledgard SF, Sprosen M, Steele K (1996) Nitrogen fixation by nine white clover cultivars in grazed pasture, as affected by nitrogen fertilization. Plant Soil 178:193–203

Liu Y, Wu L, Baddeley JA, Watson CA (2011) Models of biological nitrogen fixation of legumes. A review. Agron Sustain Dev 31: 155–172

Lloyd J, Farquhar GD (1994) ^{13}C Discrimination during CO_2 assimilation by the terrestrial biosphere. Oecologia 99:201–215

López-Bellido FJ, López-Bellido RJ, Redondo R, López-Bellido L (2010) B value and isotopic fraction in N_2 fixation by chickpea (*Cicer arietinum* L.) and faba bean (*Vicia faba* L.). Plant Soil 337: 425–434

López-Bellido RJ, López-Bellido L, Benítez-Vega J, Muñoz-Romero V, López-Bellido FJ, Redondo R (2011) Chickpea and faba bean nitrogen fixation in a Mediterranean rainfed Vertisol: effect of the tillage system. Eur J Agron 34:222–230

Masi A, Sadori L, Restelli FB, Baneschi I, Zanchetta G (2014) Stable carbon isotope analysis as a crop management indicator at Arslantepe (Malayta, Turkey) during the Late Chalcolithic and Early Bronze Age. Veg Hist Archaeobot 23:751–760

Nebiyu A, Huygens D, Ram Upadhayay H, Diels J, Boeckx P (2014) Importance of correct *B* value determination to quantified biological N_2 fixation and N balances of faba beans (*Vicia faba* L.) via ^{15}N natural abundance. Biol Fertil Soils 50:517–525

O'Leary MH (1981) Carbon isotope fractionation in plants. Phytochemistry 20:553–567

O'Leary MH (1988) Carbon isotopes in photosynthesis. BioScience 38: 328–336

Peoples MB, Brockwell J, Herridge DF, Rochester IJ, Alves BJR, Urquiaga S, Boddey RM, Dakora FD, Bhattarai S, Maskey SL, Sampet C, Rerkasem B, Khan DF, Hauggaard-Nielsen H, Jensen ES (2009) The contributions of nitrogen-fixing crop legumes to the productivity of agricultural systems. Symbiosis 48:1–17

Senbayram M, Dixon L, Goulding KWT, Bol R (2008) Long-term influence of manure and mineral nitrogen applications on plant and soil ^{15}N and ^{13}C values from the Broadbalk wheat experiment. Rapid Commun Mass Spectrom 22:1735–1740

Serret MD, Ortiz-Monasterio I, Pardo A, Araus JL (2008) The effects of urea fertilisation and genotype on yield, nitrogen use efficiency, $\delta^{15}N$ and $\delta^{13}C$ in wheat. Ann Appl Biol 153:243–257

Shearer G, Kohl DH (1986) N_2-fixation in field settings: estimations based on natural ^{15}N abundance. Aust J Plant Physiol 13:699–756

Simpson IA, Bol R, Bull ID, Evershed RP, Petzke KJ, Dockrill SJ (1999) Interpreting early land management through compound specific stable isotope analyses of archaeological soils. Rapid Commun Mass Spectrom 13:1315–1319

Stika H-P, Heiss A (2013) Plant cultivation in the bronze age. In: Fokkens H, Harding A (eds) The Oxford handbook of the European bronze age. Oxford University Press, Oxford, p 348–369

Stokes H, Müldner G, Jenkins E (2011) An investigation into the archaeological application of carbon stable isotope analysis used to establish water crop availability: solutions and ways forward. In: Mithen S, Black E (eds) Water, life and civilisation: climate, environment, and society in the Jordan valley. Cambridge University Press, Cambridge, pp 373–380

Styring AK, Fraser RA, Bogaard A, Evershed RP (2014) The effect of manuring on cereal and pulse amino acid $\delta^{15}N$ values. Phytochemistry 102:40–45

Szpak P (2014) Complexities of nitrogen isotope biogeochemistry in plant-soil systems: implications for the study on ancient agricultural and animal management practices. Front Plant Sci 5:1–19

Szpak P, Longstaffe FJ, Millaire J-F, White CD (2012a) Stable isotope biogeochemistry of seabird Gauno fertilization: results from growth chamber studies with maize (*Zea mays*). PLoS ONE 7, e33741. doi: 10.1371/journal.pone.0033741

Szpak P, Millaire J-F, White CD, Longstaffe J (2012b) Influence of seabird guano and camelid dung fertilization on the nitrogen isotopic composition of field-grown maize (*Zea mays*). J Archaeol Sci 39: 3721–3740

Szpak P, Longstaffe FJ, Millaire J-F, White CD (2014) Large variation in nitrogen isotopic composition of a fertilized legume. J Archaeol Sci 45:72–79

Tieszen LL (1991) Natural variations in the carbon isotope values of plants: implications for archaeology, ecology, and palaeoecology. J Archaeol Sci 18:227–248

Treasure ER (2014) 'Evidently carbonised by fire action': an archaeobotanical and experimental study of *Vicia faba* L. (broad bean, horsebean) in prehistoric Britain. Dissertation, Durham University

Tryderman MK, Hauggaard-Nielsen H, Jørnsgård B, Steen Jensen E (2004) Comparison of interspecific competition and N use in pea-barley, faba bean-barley and lupin-barley intercrops grown at two temperate locations. J Agric Sci 142:617–627

Unkovich M (2013) Isotope discrimination provides new insight into biological nitrogen fixation. New Phytol 198:643–646

Vaiglova P, Bogaard A, Collins M, Cavanagh W, Mee C, Renard J, Lamb A, Gardeisen A, Fraser R (2014) An integrated stable isotope study of plants and animals from Kouphovouno, southern Greece: a new look at Neolithic farming. J Archaeol Sci 42:201–215

van Klinken GT, Richards MP, Hedges REM (2002) An overview of causes for stable isotopic variations in past European human populations: environmental, ecophysiological, and cultural effects. In: Ambrose SH, Katezenberg MA (eds) Biogeochemical approaches to palaeodietary analysis. Kluwer Academic, New York, pp 39–63

Vinther F (1998) Biological nitrogen fixation in grass-clove affected by animal excreta. Plant Soil 203:207–215

Virginia RA, Delwiche CC (1982) Natural ^{15}N abundance of presumed N_2-fixing and non-N_2-fixing plants from selected ecosystems. Oecologia 54:317–325

Wallace M, Jones G, Charles M, Fraser R, Halstead P, Heaton THE, Bogaard A (2013) Stable carbon isotope analysis as a direct means of inferring crop water status and water management practices. World Archaeol 45:388–409

Watzka M, Buchgraber K, Wanke W (2006) Natural ^{15}N abundance of plants and soils under different management practices in a montane grassland. Soil Biol Biochem 38:1564–1576