Scotland's first farmers: new insights into early farming practices in North-west Europe

Rosie R. Bishop[1,7,*][ORCID: 0000-0003-1259-5374], Darren R. Gröcke[2] [ORCID: 0000-0003-2296-7530], Ian Ralston[3][ORCID: 0000-0003-1966-6665], David Clarke[4], Daniel H.J. Lee[5][ORCID: 0000-0002-3786-7025], Alexandra Shepherd[6], Antonia S. Thomas[5][ORCID: 0000-0002-1959-7260], Peter A. Rowley-Conwy[7][ORCID: 0000-0002-9494-3151] & Mike J. Church[7][ORCID: 0000-0001-7908-7812]

[1] Arkeologisk Museum, Universitetet i Stavanger, Norway

[2] Department of Earth Sciences, Durham University, UK

[3] School of History, Classics and Archaeology, University of Edinburgh, UK

[4] Independent Researcher, Edinburgh, UK

[5] Orkney College, University of the Highlands and Islands, Orkney, UK

[6] Independent Researcher, Aberdeen, UK

[7] Department of Archaeology, Durham University, UK

[*] Author for correspondence ✉ rosemary.r.bishop@uis.no

OSM1: stable isotope analysis

The stable isotope composition of archaeological crop remains provides a direct method of investigating crop growing conditions in the past, potentially allowing variation within and between harvests to be identified (Bogaard *et al.* 2013). The application of organic matter (manure, household midden, seaweed or fish remains) to crops to increase yields, may significantly increase nitrogen stable isotope values in the soil (δ^{15}N: the ratio of the stable isotopes of nitrogen, ^{15}N to ^{14}N), increasing crop stable isotope values up to *c.* +10‰ above wild plant values (Fraser *et al.* 2011; Bogaard *et al.* 2013; Blanz *et al.* 2019; Gröcke *et al.* 2021). Parameters for identifying the intensity of manuring using crop δ^{15}N values have been established from modern field trials and crops grown under traditional cultivation regimes, with higher δ^{15}N values expected for fields with high (30+ tonnes per hectare) compared to medium (<20 tonnes per hectare) or low (no manuring in the last three or more years) manuring levels (Bogaard *et al.* 2013; Styring *et al.* 2017a). Several other factors may also increase δ^{15}N values in plants to some extent, such as waterlogging, burning or naturally high soil nitrogen levels, whereas salinity may decrease δ^{15}N values in plants (Szpak 2014;

Fiorentino *et al.* 2015: 222). Further research is needed, but a recent study suggests that cereals grown on recently burned land have low $\delta^{15}N$ values (Styring *et al.* 2017b), implying that manuring and burning can be distinguished.

Thresholds have also been established for identifying levels of water availability for past crops using carbon isotope discrimination ($\Delta^{13}C$) values from modern crops grown under different irrigation regimes (Ferrio *et al.* 2005; Wallace *et al.* 2013). $\Delta^{13}C$ values are calculated from carbon stable isotope values ($\delta^{13}C$: the ratio of the stable isotopes of carbon, ^{13}C to ^{12}C), and provide a measure of how much plants avoid using—or discriminate against—^{13}C, allowing for changes in $\delta^{13}C$ in the air through time (Fiorentino *et al.* 2015). Plants preferentially utilise ^{12}C for photosynthesis, but when carbon dioxide availability is restricted (e.g. in arid conditions when stomata of plant cell walls are closed to restrict water loss) or the reaction rate is increased, insufficient ^{12}C is available and there is less discrimination against ^{13}C (Fiorentino *et al.* 2015). Thus, higher $\delta^{13}C$ values (corresponds to lower $\Delta^{13}C$ values) reflect drier growing conditions and vice versa (Wallace *et al.* 2013). Other factors which increase photosynthesis rates or reduce water availability can also result in higher $\delta^{13}C$ values (i.e. lower $\Delta^{13}C$ values) in crops, such as lower soil water-holding capacities or increased temperatures or light intensities (Heaton 1999). An increase in soil salinity will also cause plants to reduce their stomata apertures, resulting in less discrimination against ^{13}C and higher $\delta^{13}C$ values (i.e. lower $\Delta^{13}C$ values; Gröcke 1998, 2002).

OSM2: stable isotope sample selection and analysis

A total of 196 Neolithic cereal grains and 10 crab apple seeds were selected for stable isotope analysis. We selected key contexts from each site for analysis on the basis that they contained concentrations of well-preserved cereal grains of one or more crop species. The degree of cereal grain preservation was determined using the preservation scale of Boardman & Jones (1990) and only grains in the P1–P3 range were selected for analysis to minimise isotopic offsets caused by charring at high temperatures. An adapted scale was used to record the preservation of the crab apple seeds, based on the degree of seed coat surface covering (see Table S6). Overall, 76.5 per cent of the grains and 100 per cent of the crab apple seeds were very well preserved, falling in the P1 and P2 preservation categories, with a further 23.5 per cent of the grains falling in the P3 preservation category. Where more than one crop species was present in the selected context, we analysed grains from both species to examine any differences in crop husbandry practices between species. Where possible, 10 grains or seeds

were analysed from each selected context, though in some cases a smaller number were selected due to the low numbers of grains present and the need to preserve grains for future analyses.

Each grain and seed was individually analysed for stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) to examine the level of variability within contexts, whether there was any relationship between the size of the grain and the stable isotope content, and to reduce the number of archaeological grains destroyed as part of the analyses. Prior to analysis the length, width and depth of each grain and seed was measured to the nearest 0.1mm using the internal graticule of a Leica M80 stereomicroscope under $\times$ 7.5–60 magnification and the mass of each grain was measured to the nearest 0.001g using a Mettler PM480 Delta Range balance. Grains with adhering sediment were avoided for analysis, but where necessary grains were carefully scraped with a clean scalpel to remove any adhering sediment to ensure reliable $\delta^{13}C$ and $\delta^{15}N$ results were produced (Brinkkemper *et al.* 2018). Prior to analysis, each grain was crushed using a pestle and mortar. No further pre-treatment of grains was undertaken. Stable isotope analyses, total organic carbon and total nitrogen content were determined using a Costech Elemental Analyser (ECS 4010) connected to a Thermo Scientific Delta V Advantage isotope ratio mass spectrometer in the Stable Isotope Biochemistry Laboratory (SIBL) at Durham University. Carbon isotope ratios were corrected for ^{17}O contribution and reported in standard delta (δ) notation in per mil (‰) relative to Vienna Pee Dee Belemnite (VPDB). Isotopic accuracy was monitored through routine analyses of in-house standards (Glutamic acid, $\delta^{13}C = -11.00‰$, $\delta^{15}N = -7.50‰$; Urea, $\delta^{13}C = -44.00‰$, $\delta^{15}N = 0.00$; Spar Calcite, $\delta^{13}C = +2.90‰$), which were stringently calibrated against international standards (e.g. USGS40, USGS24, IAEA-600, IAEA-N-1, IAEA-N-2): this provided a linear range in $\delta^{13}C$ between −44‰ and +3‰ and in $\delta^{15}N$ between −7.5‰ and +20.4‰. Analytical variation in carbon and nitrogen isotope analyses was typically ±0.1‰ for replicate analysis of the international standards and typically <0.2‰ on replicate sample analysis. BOH S.41 IS5 and IS6 produced unusually high $\delta^{15}N$ values (14.19‰ and 9.05‰ respectively before adjusting for charring) and therefore these samples were subsequently reanalysed and a consistent result was produced (14.02‰ and 9.06‰, respectively, before adjusting for charring). Total organic carbon and nitrogen data was obtained as part of the isotopic analysis using an internal standard (Glutamic Acid, 40.82% C, 9.52% N). Stable isotope results were adjusted for potential charring offsets by subtracting 0.11‰ from $\delta^{13}C$ and 0.31‰ from $\delta^{15}N$ (Nitsch *et al.* 2015). $\delta^{13}C$ values of atmospheric CO_2 for each context were established using the

radiocarbon dates for each site and the AIRCO2_LOESS system (Ferrio *et al.* 2005). $\Delta^{13}C$ values were calculated following the methodology of Farquhar *et al.* (1989).

The $\delta^{15}N$ baseline for wild unmanaged plants in Orkney (2.1‰) was estimated by subtracting the mean offset for herbivore diets (4‰) (Bogaard *et al.* 2013) from the mean Neolithic herbivore bone collagen $\delta^{15}N$ for Orkney (6.1‰) (Schulting & Richards 2009; Jones & Mulville 2016; Schulting *et al.* 2017). For the mainland sites (Balbridie and Dubton Farm), no relevant Neolithic herbivore bone collagen $\delta^{15}N$ values were available due to acidic soil conditions and so the wild unmanaged plant baseline was estimated using a mean of the $\delta^{15}N$ for the Crab Apple (*Malus sylvestris* (L.) Mill.) seeds from Dubton Farm (0.9‰) because crab apple is generally considered to have been a wild uncultivated tree in prehistory. Quartile calculations are shown exclusive of the median.

Crop $\delta^{15}N$ and $\delta^{13}C$ stable isotope values was synthesised for other Neolithic sites in north-west Europe (Figure 8) from the following sources: England and Wales (Bogaard *et al.* 2013; Treasure *et al.* 2019), Denmark and Sweden (Bogaard *et al.* 2013; Kanstrup *et al.* 2014; Gron *et al.* 2017, 2021) and Germany (Bogaard *et al.* 2013; Styring *et al.* 2016, 2017b; Filipović *et al.* 2019). Data was included for sites dated to the Neolithic period for each country within the chronological range 5500–2500 cal BC (data from sites/samples dating to *c.* 2500–2000 cal BC excluded). Data points from Table S5, Gron *et al.* (2017), Treasure *et al.* (2019) and Gron *et al.* (2021) are individual grains, whereas data points from Bogaard *et al.* (2013), Kanstrup *et al.* (2014) and Styring *et al.* (2016, 2017b) consist of bulk samples of approximately 5–10 cereal grains. All stable isotope results were adjusted for potential charring offsets by subtracting 0.11‰ from $\delta^{13}C$ and 0.31‰ from $\delta^{15}N$ (Nitsch *et al.* 2015). +1‰ was added to the crop $\delta^{15}N$ values from Bogaard *et al.* (2013) because the raw values in the publication had been adjusted by subtracting one to allow for charring, and it has subsequently been shown that it is only necessary to subtract 0.31‰ to correct for charring (Nitsch *et al.* 2015).

OSM3: archaeobotanical methods

The cereal assemblage composition charts in Figure 3 include data for the contexts analysed in the stable isotope study from Dubton Farm (Contexts B215, B037/1, B233/1, B187; Church 2002), Balbridie (F40 and F294; Fairweather & Ralston 1997), Skara Brae (Context 168; Shepherd 2016; Rowley-Conwy & Bishop 2021) and the Braes of Ha'Breck (Contexts 418, 634, 414, 427, excluding C.1104 and C.197, which are currently under analysis; Bishop 2013). The wild seed data for each site were summarised on a presence/absence level only

because the final archaeobotanical reports for three of the assemblages (Balbridie, Braes of Ha'Breck and Skara Brae) have not yet been published (see Table S1). The aim was to provide an impression of the overall ecological character of all the wild seed taxa for each site and hence the data were considered on an assemblage rather than sample basis and thresholds for numbers of identifications per sample were not set. The crop compositional data, seed ecological data and context associations for the analysed assemblages from Balbridie and Dubton Farm suggest that the wild seed taxa are 'weed seeds' associated with the arable crops (Fairweather & Ralston 1997; Church 2002). In contrast, the higher proportion of freshwater/heathland taxa and wet ground indicators (e.g. *Carex* sp.) in the Braes of Ha'Breck assemblage suggests that a mix of 'weed seeds' and seeds deriving from the burning of peaty turf are represented (Bishop 2013; see also Church *et al.* 2007). The Skara Brae wild seed assemblage post-dates the cereal crop assemblage and may consist mainly of peaty turf burning debris (Rowley-Conwy & Bishop 2021): it is included here as an illustration of the range of wild taxa recovered at the site and to highlight similarities with the Braes of Ha'Breck assemblage. Further experimental investigation is needed to disentangle these data, which is beyond the scope of the present study. Habitat and perennation type were classified for the wild seed taxa using ecological information from Hill *et al.* (2008), with the general habitat categories following Bogaard & Jones (2007) (see Table S2). No threshold was set for the quantity of wild seed taxa per sample or site phase as this was generally low at Balbridie and the Braes of Ha'Breck, where fully processed crops may be represented. Tree and shrub taxa were excluded from the ecological assessment of wild seed data as they do not grow as weeds in arable fields. Only seeds with species or genus-level identifications with clear habitat associations were included in the ecological analyses (see Table S2). The *Prunella* sp. identification was classified using ecological information for *Prunella vulgaris* L. (Selfheal), as this is the only species of *Prunella* native to Scotland (Stace 2019). Nomenclature follows Stace (2019) for the wild plants and the cereals.

Table captions

Table S1: Summary of wild seed taxa for study sites (Fairweather & Ralston 1997; Church 2002; Bishop 2013; Rowley-Conwy & Bishop 2021). P = present.

Table S2: List of wild seed taxa for study sites and their corresponding habitat associations and perennation type (archaeobotanical data: Fairweather & Ralston 1997; Church 2002; Bishop 2013; Rowley-Conwy & Bishop 2021). a = annual; p = perennial.

Table S3: Summary of crop nitrogen and carbon stable isotope values, grain size measurements and preservation categories for cereals in the stable isotope study.

Table S4: Summary of crop nitrogen and carbon stable isotope values by feature for cereals in the stable isotope study.

Table S5: Crop nitrogen and carbon stable isotope values, grain and seed size measurements and preservation categories for cereals and seeds in the stable isotope study.

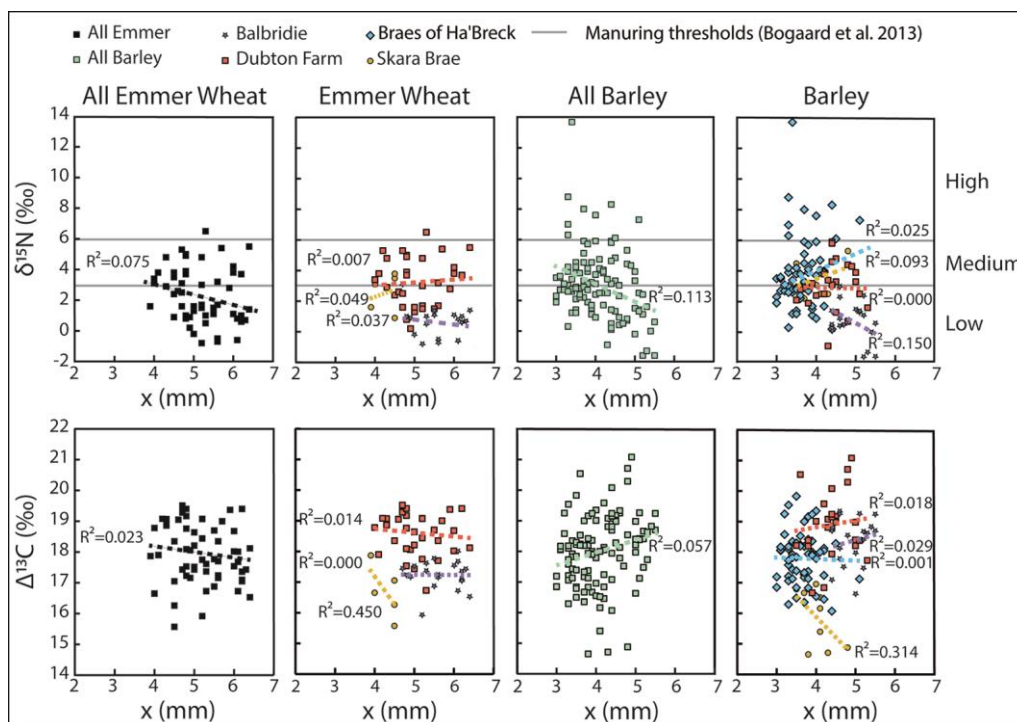**Table S6: Preservation scale used for recording the crab apple seeds.**

Figure S1. Grain size measurements plotted against crop nitrogen and carbon stable isotope values for cereals in the stable isotope study.

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