Understanding variability in $\delta^{15}N$ and $\delta^{13}C$ values of single grains from bread wheat

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Introduction

Isotopic analysis of archaeological plant remains has, until now, focused predominantly on the analysis of bulk samples of seeds, commonly with the sample containing between five and 30 individual seeds (e.g. Bogaard *et al*. 2013; Nitsch *et al*. 2015; Styring *et al*. 2017). This approach results in the averaging of the isotopic variability of individual seeds, allowing for an understanding of the overall isotopic value of a context. Modern experimental data used to interpret archaeological data have also been based principally on bulk samples. However, due to developments in mass spectrometry, it is now possible to measure smaller amounts of nitrogen, using smaller samples, and thus allowing the analysis of single seeds. The analysis of single seeds has in turn opened up isotopic analysis to mixed deposits, whereas in the past this was confined to *in situ* storage deposits of large quantities of seeds. The change from bulk to single grain analysis requires the development of a framework for the interpretation of single grain isotopic data: in particular, an understanding of both the variability in $\delta^{13}C$ and $\delta^{15}N$ values, and how much variability would be expected in single grains from the same field.

There has been some previous work examining how variable single grains are within the cereal ear, as well as within a single field (see Table 1). Research was recently conducted by Larsson *et al*. (2019) which produced within-ear and within-plot data on barley grain $\delta^{15}N$ values for both manured (25 tonnes per hectare of farm slurry from cattle) and non-manured plants. The present report contains the results of a complementary analysis of bread wheat from low-level manured fields, providing information on the variability within ear, plant, 1m^2 quadrat and field.

Table 1 – Previous work on grain variability.

Unit	$\delta^{13}C$	$\delta^{15}N$
Ear	Standard deviation ±0.6‰ (Heaton *et al*. 2009)	1.8±1‰ (Bogaard *et al*. 2007) Non-manured: ~5.4±0.15 (Larsson *et al*. 2019) Manured: ~8.75±0.26 (Larsson *et al*. 2019)
Plant	Only bulk data	None published
Field	Standard deviation range 0.32-0.67‰ (Heaton *et al*. 2009)	Non-manured: 5.39±0.56 (Larsson *et al*. 2019) Manured: 8.87±1.64‰ (Larsson *et al*. 2019)

Experimental design

The experiment was designed to examine variability within the ear, plant and 1m^2 quadrat as well as the overall variability in a single field. The isotopic samples were taken from the location of previous weed survey quadrats to allow for future comparisons between weed data and isotopic data. The quadrats were evenly spaced along an accessible transect of the field, at least a metre or more into the crop to avoid edge effects. Quadrat 1 was sampled for whole plants to allow for the selection of ears from different tillers of the same plant. Three plants with three or more tillers (with ears) were selected for use, with three ears per plant chosen for analysis and 90 grains within those ears selected (see Table 2). From the remaining four quadrats, ten grains from random ears were selected. In total, 130 single grains were analysed for both stable carbon and nitrogen isotope measurements (Table 3).

Table 2 – The organisation of the samples from Quadrat 1.

Quadrat 1								
Plant 1			Plant 3			Plant 4		
ear 1	ear 2	ear 3	ear 1	ear 2	ear 3	ear 1	ear 2	ear 3
10 samples	10 samples	10 samples	10 samples	10 samples	10 samples	10 samples	10 samples	10 samples

Table 3 – The number of items and grains sampled.

Number of items	Number of grains
9 ears	10 per ear
5 quadrats	10 per quadrat 2,3,4,5
3 plants	30 per plant

The samples were collected on 30th July 2019 from a field at the Duchy of Cornwall's Home Farm, Highgrove, Gloucestershire. The material used in this study came from the upper section of the Beverston, named field 8 (HIGH08) in the Highgrove weed survey. The field was approximately 10 acres in size and was sown with a heritage variety of bread wheat called Maris Wigeon. Home Farm is organic, and practises a seven-year rotation system which includes two to three years of fallow in the form of grass/red clover. Composted farmyard manure is added at the end of the fallow period, then a cropping system is used, consisting of winter wheat followed by oats, then spring barley and rye as the final crop.

Isotope methods

The samples were prepared at the University of Oxford for simultaneous carbon and nitrogen determination at Iso-Analytical Limited on a Europa Scientific 20-20 IRMS. Every tenth sample was duplicated to understand precision, while calibration standards of IAEA N1, N2, CH6 and CH7, as well as IA-R005, IA-R006, IA-045 and IA-046 (in-house Iso-Analytical standards), were included. Furthermore, check standards of EMA-P2 and IA-R001 were used to understand accuracy. Precision of the $\delta^{13}C$ values was ±0.03‰, while accuracy was ±0.14‰. The precision of the $\delta^{15}N$ values was ±0.06‰ and the accuracy was ±0.16‰. Total overall uncertainly as per Szpak *et al*. (2017) was ±0.14‰ for $\delta^{13}C$ values and ±0.17‰ for $\delta^{15}N$ values.

Results

Within-ear differences

Table 4 shows the mean, standard deviation, and variance of the single grain samples from three ears per plant in Quadrat 1, while Figure 1 shows the data graphically. Within-ear $\delta^{13}C$ values range from -26.67‰ to -25.54‰, with ear 1 from plant 1 having the widest range, a difference of 0.83‰ from maximum to minimum value. The grains from ear 1 of plant 4 have the smallest range of values: a difference of 0.26‰ from maximum to minimum. It is notable that the $\delta^{13}C$ values of ear 3 of plant 1 and some of the samples from ear 1 are different from overall values found in ear 2, as well as all ears in plant 3 and 4 (Figure 1). Within-ear $\delta^{15}N$ values range from -0.1‰ to 1.99 ‰, with ear 1 of plant 4 having the biggest difference between maximum and minimum values, of 0.52‰. Ear 3 of plant 1 has the narrowest range and smallest difference between maximum and minimum values, of 0.15‰. It is notable that the mean $\delta^{15}N$ value of ear 3 of plant 3 (1.84‰) is different from ear 1 and 2 (1.24‰ and 1.31‰ respectively) by more than 0.5‰.

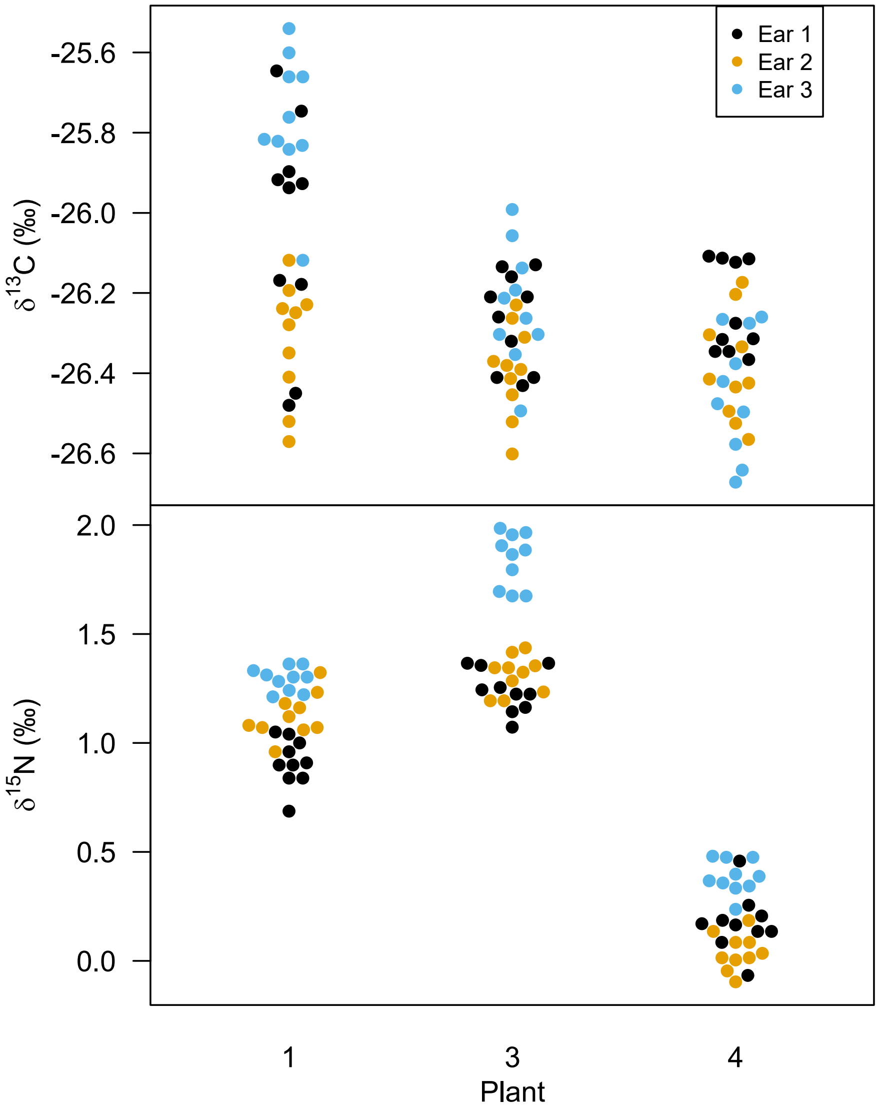

Figure 1 – The $\delta^{13}C$ and $\delta^{15}N$ values of the individual grains from three ears from three different plants.

Table 4 – Isotopic results of single grains from ears of three plants from a single quadrat.

			$\delta^{13}C$ (‰)						$\delta^{15}N$ (‰)					
Plant	Ear	No of grains	Mean	Variance	Standard deviation	Max	Min	Max-min	Mean	Variance	Standard deviation	Max	Min	Max-min
1	1	10	-26.03	0.08	0.28	-25.65	-26.48	0.83	0.91	0.01	0.11	1.05	0.69	0.36
1	2	10	-26.32	0.02	0.15	-26.12	-26.57	0.45	1.13	0.01	0.10	1.32	0.96	0.36
1	3	10	-25.77	0.03	0.16	-25.54	-26.12	0.58	1.29	0.00	0.05	1.36	1.21	0.15
3	1	10	-26.27	0.01	0.12	-26.13	-26.43	0.30	1.24	0.01	0.10	1.37	1.07	0.29
3	2	10	-26.39	0.01	0.11	-26.23	-26.60	0.37	1.31	0.01	0.09	1.44	1.19	0.24
3	3	10	-26.23	0.02	0.15	-25.99	-26.49	0.50	1.84	0.01	0.12	1.99	1.67	0.31
4	1	10	-26.24	0.01	0.11	-26.11	-26.37	0.26	0.17	0.02	0.13	0.46	-0.07	0.52
4	2	10	-26.39	0.02	0.13	-26.17	-26.57	0.39	0.04	0.01	0.08	0.19	-0.10	0.28
4	3	10	-26.45	0.02	0.15	-26.26	-26.67	0.41	0.39	0.01	0.08	0.48	0.24	0.24

Table 5 – The $\delta^{13}C$ and $\delta^{15}N$ mean values, standard deviation and maximum and minimum values shown by plant.

		$\delta^{13}C$ (‰)						$\delta^{15}N$ (‰)					
Plant	No. of grains	Mean	Variance	Standard deviation	Max	Min	Max-min	Mean	Variance	Standard deviation	Max	Min	Max-min
1	30	-26.04	0.09	0.30	-25.54	-26.57	1.03	1.11	0.03	0.18	1.36	0.69	0.68
3	30	-26.30	0.02	0.14	-25.99	-26.60	0.61	1.46	0.08	0.29	1.99	1.07	0.91
4	30	-26.36	0.02	0.16	-26.11	-26.67	0.56	0.20	0.03	0.17	0.48	-0.10	0.58
1	10	-25.97	0.07	0.27	-25.66	-26.41	0.75	1.15	0.03	0.16	1.36	0.91	0.45
3	10	-26.29	0.02	0.15	-26.06	-26.60	0.54	1.51	0.13	0.36	1.99	1.14	0.84
4	10	-26.36	0.04	0.20	-26.11	-26.67	0.56	0.21	0.04	0.21	0.48	-0.07	0.54

Table 6 – The $\delta^{13}C$ and $\delta^{15}N$ mean values, standard deviation and maximum and minimum values shown by quadrat.

		$\delta^{13}C$ (‰)						$\delta^{15}N$ (‰)					
Quad	No. of grains	Mean	Variance	Standard deviation	Max	Min	Max-min	Mean	variance	Standard deviation	Max	Min	Max-min
1	90	-26.23	0.06	0.25	-25.54	-26.67	1.13	0.93	0.33	0.58	1.99	-0.10	2.08
1	10	-26.14	0.1	0.31	-25.54	-26.58	1.04	0.90	0.39	0.62	1.87	0.08	1.78
2	10	-25.78	0.06	0.25	-25.50	-26.36	0.86	-1.39	0.01	0.10	-1.23	-1.59	0.36
3	10	-26.42	0.02	0.16	-26.19	-26.65	0.45	-0.51	0.01	0.08	-0.39	-0.61	0.22
4	10	-26.43	0.04	0.20	-26.10	-26.74	0.64	-0.59	0.01	0.09	-0.45	-0.71	0.26
5	10	-26.01	0.02	0.13	-25.81	-26.15	0.34	0.17	0.01	0.08	0.33	0.08	0.25

Within-plant differences

The $\delta^{13}C$ values of plant 1 (-26.04±0.3‰) are variable, ranging from -25.54‰ to -26.57‰ (a difference of 1.03‰) (Table 5). This is due to the wide range of values in the ears of plant 1 as well as the difference in mean values between ear 2 (-26.12‰) and ear 3 (-25.77‰) (Figure 1). Plant 3 (-26.3±0.14‰) has a difference between the maximum and minimum values of 0.61‰, while for plant 4 (-26.36± 0.16‰) it is 0.56‰. The $\delta^{15}N$ values of plants 1 (1.11±0.18‰) and 4 (0.2±0.17‰), however, are less variable than those of plant 3 (1.46±0.29‰) which are more variable due to the elevated values of ear 3. Plants 1 and 4 have similar differences between their maximum and minimum values (0.68‰ and 0.58‰ respectively), while plant 3 has a difference of 0.91‰.

Due to the nature of the experiment, the comparison between the ear data and plant data uses different numbers of grains: ten grains per ear, summed to 30 grains per plant. To test the difference that might occur, a random ten-grain subsample of the 30 grains was taken per plant (Table 5), revealing a slight reduction in the standard deviations. The $\delta^{13}C$ standard deviation for plant 1 is reduced, with the difference between the maximum and minimum values also reduced to 0.75‰ (Table 5). The $\delta^{13}C$ ranges and standard deviations of the other plants show limited change. For $\delta^{15}N$, the ten-grain subsamples result in limited change to the standard deviation and a small reduction in the maximum to minimum differences for each plant.

Between-plant differences

The $\delta^{13}C$ means of the three plants are similar (Table 5), with a difference of 0.32‰ between the mean of plant 1 (-26.04±0.3‰) and that of plant 4 (-26.36±0.16‰). The standard deviations of the three plants are within 0.16‰ of each other. The impact of sub-sampling just ten grains per plant increases the difference between the three plants to 0.39‰ but reduces the difference between their standard deviations (0.13‰). The $\delta^{15}N$ values of plants 1 and 3 are similar, while the $\delta^{15}N$ mean of plant 4 is lower by 1.26‰ (Table 5; Figure 1). The standard deviations of the three plants are within 0.11‰ of each other, with plant 3 having the highest standard deviation (±0.29‰) because of the high $\delta^{15}N$ values from ear 3 as noted above. Again, to test the impact of different grain numbers in comparison to the ear data, a ten-grain subsample was taken, resulting in a small change to the standard deviation (0.2‰ difference between standard deviations) and increases the difference between the means (±1.3‰).

Quadrat differences

The mean $\delta^{13}C$ values of the five quadrats range from -26.19‰ to -25.5‰, with the standard deviation of each quadrat's values around 0.2‰ (Table 6). The ranges within the five quadrats are greater than those within and between ear and plant, with Quadrat 1 having the largest difference between the maximum and minimum value (1.13‰). However, it must be remembered that Quadrat 1's data are from a total of 90 grains, while the data from Quadrats 2, 3, 4 and 5 are from ten random single grains. To make the comparison even, ten random grains were selected from Quadrat 1, producing a difference between the maximum and minimum values of 1.04‰, which is more in line with the other quadrats (Table 6). There are some differences between the quadrats: Quadrat 2 has the highest mean at -25.5‰, a value similar to the mean of plant 1 from Quadrat 1 (Figure 2).

The mean $\delta^{15}N$ values of the quadrats are variable, ranging from -1.39‰ to 0.93‰. Quadrat 1 has the highest mean value and is also the most variable, ranging from -0.1‰ to 1.99‰, a 2.1‰ difference.

Conversely, the other quadrats have smaller differences between their maximum and minimum values (0.22‰ to 0.36‰) (Table 6). The high variability in $\delta^{15}N$ values is notable for Quadrat 1 (see Figure 2), with the variability due not only to the differences between the three plants within that quadrat, but also to the more enriched overall values found in all samples from Quadrat 1. Again, to ensure that the comparison between quadrats was based on the same number of grains, a random sample of ten grain values was taken, producing a high standard deviation of 0.62 and range difference of 1.78‰ for Quadrat 1. This is still more variable than the other quadrats by ~ 1.5‰ for the max-min value.

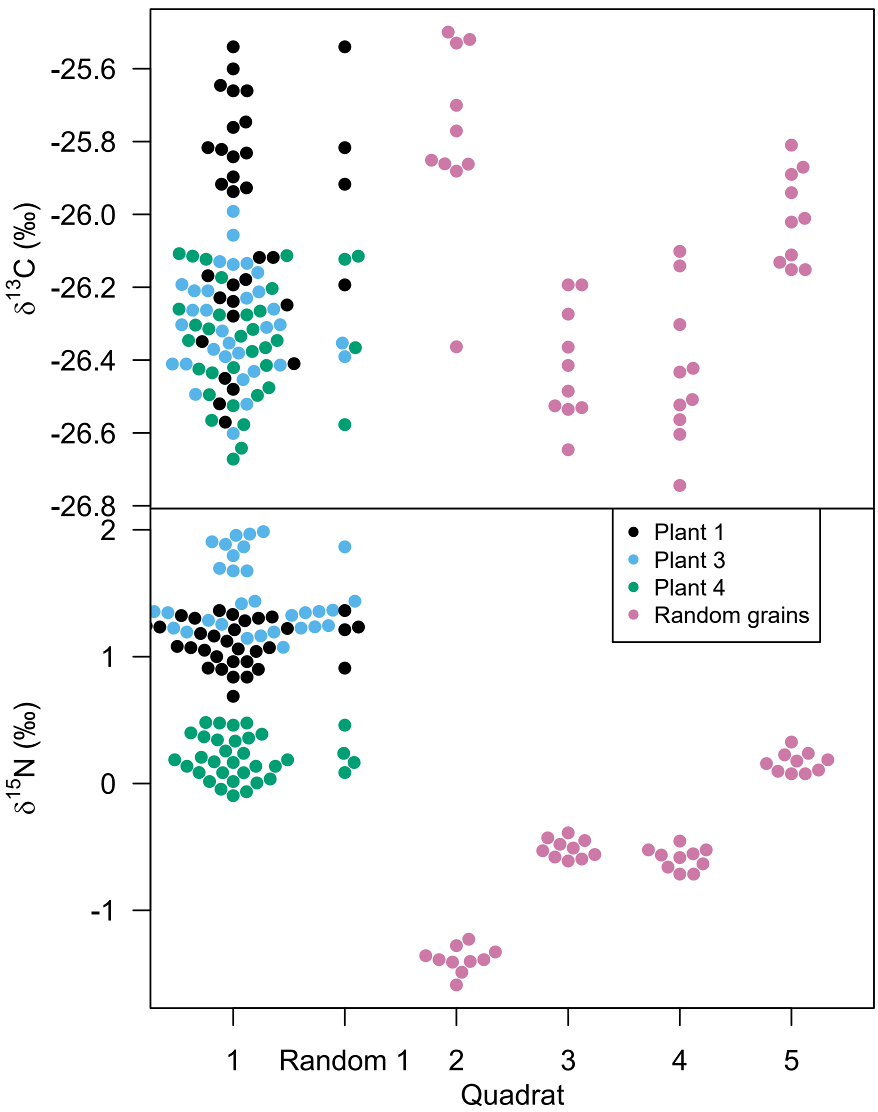

Figure 2 – The isotopic values for single grains from Quadrats 1, 2, 3, 4 and 5, as well as the randomly selected ten grains from Quadrat 1 (labelled Random 1).

Field values

The overall values for the field were calculated from the entire samples (Table 7), with a mean $\delta^{13}C$ value of -26.21±0.28‰ and a difference from the maximum to minimum of 1.24‰. As Quadrat 1 has significantly more data points, the overall field value was also calculated as an average of the ten random grains per quadrat (50 grains), as well as the averages of just ten grains randomly selected from the 50 random grains (Table 7). While the ten-grain grab does have a reduced maximum-minimum value, it is only reduced by 0.2‰.

The $\delta^{15}N$ mean of the field is 0.46±0.9‰ when 130 grains are used, with a difference between the maximum and minimum of 3.57‰. The mean changes for the different grabs of samples, and the standard deviation decreases with sample size. The ten-grain grab produces the smallest difference between the maximum and minimum values, of 1.78‰, a reduction of more than 1.7‰ from the 130-grain batch.

Table 7 – The mean, variance, standard deviation, maximum, minimum and max-min difference of the $\delta^{13}C$ and $\delta^{15}N$ values from the 130 samples, average of ten grains per quadrat, and a random ten grains.

	$\delta^{13}C$ (‰)						$\delta^{15}N$ (‰)					
No. of grains	Mean	Variance	Standard deviation	Max	Min	Max-min	Mean	Variance	Standard deviation	Max	Min	Max-min
130	-26.21	0.08	0.28	-25.50	-26.74	1.24	0.46	0.81	0.90	1.99	-1.59	3.57
50	-26.16	0.11	0.33	-25.50	-26.74	1.24	-0.29	0.68	0.82	1.87	-1.59	3.45
10	-26.14	0.10	0.31	-25.54	-26.58	1.04	0.90	0.39	0.62	1.87	0.09	1.78

Discussion and implications

The majority of the maximum to minimum differences for the within-ear and within-plant data are under 1‰ (Tables 4–5). Comparison with other published data shows that the within-ear standard deviations of the Highgrove data's $\delta^{13}C$ values (±0.11‰ to ±0.28‰) are lower than the standard deviation published by Heaton *et al*. (2009) (±0.6‰). The standard deviation of the $\delta^{15}N$ values of the Highgrove data (±0.05‰ to ±0.13‰) are much lower than the Bogaard *et al*. (2007) ±1‰ standard deviation, and closer to the standard deviations obtained by Larsson *et al*. (2019), in particular from the non-manured samples.

The ear $\delta^{15}N$ values from Highgrove fall between Larsson *et al*.'s (2019) two groups (Table 1). Highgrove fields received low levels of manuring, ~ 3-5 tons per acre (composted farmyard manure). It is also notable that the farm cultivates its crops in rotation, with one of the rotations a forage/silage crop of red clover. It is possible that the $\delta^{15}N$ values of the cereal crop are low partly as a result of this.

The within- and between-plant results highlight that variability in $\delta^{13}C$ and $\delta^{15}N$ values is in part due to a combination of differences between-ear, within-plant and between-location within a field. The between-plant data show that plant $\delta^{15}N$ values can differ by as much as 1.2‰, even when occurring within a 1 x 1 metre square (plant 3 vs plant 4 $\delta^{15}N$ values). Variability in $\delta^{13}C$ values appears to be more of a consequence of difference between and within ears in this study, and it is notable that the higher $\delta^{13}C$ values of ear 3 in plant 1 are very similar to the overall $\delta^{13}C$ values of Quadrat 2.

Causes of the $\delta^{13}C$ and $\delta^{15}N$ variability most likely relate to different soil water availability and variability in $\delta^{15}N$ soil values respectively. The more enriched $\delta^{15}N$ values of Quadrat 1, and to a lesser degree Quadrat 5, are hypothesised to be due to the proximity of these quadrats to the gate entrance to the field. FYM is stored in the field prior to spreading, with the second and third (if needed) turning of the manure (to allow in oxygen) occurring within the field. It is highly likely that manure was stored close to the gate before spreading, leading to these locations being more enriched in ^{15}N. Variability in the $\delta^{13}C$ values could be explained by different soil conditions. Research by Peukert *et al*. (2012) shows that bulk soil $\delta^{13}C$ values from a 75 x 75m area range from -28.13±0.64‰ to -26.86±0.88‰, with the higher values coming from soil with a higher soil organic matter, and different soil type than the latter, highlighting how soil conditions may influence $\delta^{13}C$ values in small areas.

The results from the study at Highgrove highlight the inherent variability in isotopic values within a wheat ear, plant, 1m^2 quadrat and field. For $\delta^{13}C$ measurements, a standard deviation of ±0.33‰ or less would be consistent with grains from a single field (Table 7). For $\delta^{15}N$ measurements a standard deviation of ±0.9‰ would be consistent with grains from a single field. Variability in isotope values seems to be related to the variability in spreading and storing of manure, which could be affected by the size of fields and method of transportation and spreading. Moreover, given that Highgrove is considered a low-level manured site, it is hypothesised that in highly manured conditions such variability would likely increase due to inconsistencies in the spatial intensity of manure application.

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