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Manuring and stable nitrogen isotope ratios in cereals and pulses: towards a new archaeobotanical approach to the inference of land use and dietary practices

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Abstract

This paper explores the impact of animal manure application on the $\delta^{15}N$ values of a broad range of crops (cereals and pulses), under a range of manuring levels/regimes and at a series of locations extending from northwest Europe to the eastern Mediterranean. We included both agricultural field experiments and areas where 'traditional' farming is practised. Our aim is to ground-truth interpretation of $\delta^{15}N$ values in archaeobotanical crop remains as evidence of past growing conditions and husbandry practices. The results confirm the potentially radical impact of manuring on $\delta^{15}N$ values in cereals, depending on manuring level, but indicate only a slight effect on pulses, which can fix atmospheric nitrogen. The expected geographical trend towards greater $\delta^{15}N$ with increasing climatic aridity is not apparent, probably because the growing conditions for crops are 'buffered' through crop management. Each of these observations has fundamental implications for archaeobotanical interpretation of $\delta^{15}N$ values as evidence of land use practices and (together with analysis of bone collagen/tooth enamel in potential consumers) palaeodiet.

1. Introduction

Human land use and cultivation practices for food and fodder production have altered the natural nitrogen (N) cycle in many terrestrial ecosystems. Changes in stable nitrogen isotope ratios ($^{15}N/^{14}N$ ratios expressed as $\delta^{15}N$ values) in soils and plants have been used to trace anthropogenic influences on the N cycle at different temporal and spatial scales (Koerner et al. 1999; Robinson 2001; Templer et al. 2007; Watzka et al. 2006). One of the most influential effects on soil N dynamics within agricultural systems is the use of animal manure to improve overall soil fertility (Bol et al. 2008; Choi et al. 2006; Senbayram et al. 2008). This practice has been observed to result in an increase in the $\delta^{15}N$ value of soil, primarily

due to bacterially-mediated reactions where the lighter ^{14}N isotope is preferentially lost leading to ^{15}N enrichment within the residual soil mineral N pools available to plants (Heaton 1986; Kendall et al. 2007; Kreitler and Jones 1975). Measures to improve soil conditions for crop production are important for understanding the development of agriculture. We seek to extend the use of natural abundance $\delta^{15}N$ studies to explore the nature of early farming by examining the isotopic composition of archaeobotanical remains.

Whilst a wide variety of natural environmental factors have been found to influence the $\delta^{15}N$ values of plants (climate, water stress, salinity and soil processes, particularly mineralization and denitrification), we concentrate here on the effect of fertilization with animal manure, which is associated with marked enrichment of ^{15}N in soils and plants (Bol et al. 2005; Handley et al., 1999a, Heaton ,1987; Inglett et al., 2007; Yousfi et al., 2010). A recent pilot study (Bogaard et al. 2007) on the impact of manuring on modern cereal $\delta^{15}N$ values in two long-term agricultural experiments, one in the UK and the other in Germany, showed that manuring raised crop $\delta^{15}N$ values by 2.6 to 8.0 ‰, depending on intensity, compared to the unmanured fields at the same sites. Experimental charring as part of this study suggested further that manuring is potentially detectable through $\delta^{15}N$ values in charred archaeobotanical crop remains.

Direct identification of manuring practices from the remains of ancient crops would enhance the reliability and resolution of inferences on both land use practices and palaeodiet. With regard to manuring as part of land use, other archaeobotanical evidence is more ambiguous. Weed seeds associated with crops generally reflect overall growing conditions rather than manuring *per se* (e.g., Bogaard 2004: 142; Jones 2002) and their inclusion would complement the approach considered here by excluding other potential causes of $\delta^{15}N$ variability. Chemical and micromorphological analysis of rarely preserved palaeosols may indicate the deposition of manure but not necessarily the crops concerned or the intensity of manuring (Bull et al. 1999; Canti 1997; Evershed et al. 1997; Guttmann 2005; Simpson et al. 1999).

With regard to palaeodiet, the $\delta^{15}N$ values of soils and plants are passed on up though the food chain and recorded in the isotopic signatures of various soil invertebrates, herbivores and eventually human diets. Modern isotope studies observe increases in $\delta^{15}N$ values, of

approximately 2 to 4‰, with each trophic step up the food chain (Ambrose 2000; Schoeninger and DeNiro 1984). Hence, the $\delta^{15}N$ values in human and animal remains are central to the inference of trophic level in palaeodietary studies (Lee-Thorp 2008; Styring et al. 2010). A number of authors have noted that plant $\delta^{15}N$ values vary considerably, potentially complicating conventional interpretation of $\delta^{15}N$ values in faunal and human remains as reflecting the balance of animal- versus plant-derived foods in the diet (Dürrwächter et al. 2006; Hedges and Reynard 2007; Katzenberg 2000; van Klinken et al. 2000). If the animals and plants consumed by humans have similar $\delta^{15}N$ values, for example +7‰, a problem of equifinality exists since it is impossible to distinguish a carnivorous or vegetarian diet from human values of approximately +10‰. Information on $\delta^{15}N$ in plants is therefore essential for reliable interpretation and should ideally be evaluated alongside animal and human remains (e.g., Katzenberg 2000; van Klinken et al. 2000: 39-63). The evident impact of manuring on at least some food plants demonstrates that the interpretation of palaeodiet must be placed in a broader context of land use.

In this study we build on previous results in three ways: 1. by exploring the impact of animal manure application on $\delta^{15}N$ values in a broader spectrum of crops, both cereals and pulses; 2. by considering a wider range of manuring levels; and 3. by encompassing study locations extending from northwest Europe to the eastern Mediterranean. The present study includes agricultural long-term experiments running for more than a century (Rothamsted, UK; Askov, Denmark; Bad Lauchstädt, Germany) and short-term experiments established as part of the project (at Sutton Bonington, UK and Tal Jebeen near Aleppo, Syria). Fields under 'traditional' farming regimes previously surveyed for their associated weed floras with a view to indirect archaeobotanical inference of crop husbandry practices were also included (Charles et al. 2002; Hajnalová et al. 2007; Jones et al. 1999, 2000). The aim was to facilitate interpretation of $\delta^{15}N$ values in archaeobotanical crop remains as evidence of land management and as a key part of palaeodietary investigations in Europe and southwest Asia. To this end, in addition to grains of cereals and seeds of pulses, we also analysed a further plant part identifiable to species and widely preserved in charred form: the rachis (central axis of the cereal ear), which in free-threshing cereals (such as bread wheat and barley) is separated from other chaff elements by threshing and in glume wheats (einkorn, emmer and spelt) retains the robust glumes.

Whereas the previous study by Bogaard et al. (2007) considered only one cereal species (bread wheat, *Triticum aestivum* L.), the present study was designed to assess manuring impact on a range of cereal species and also on pulses. Pulses are harvested from leguminous plants which host bacteria capable of N_2-fixation: the conversion of atmospheric N_2 ($\delta^{15}N$ = 0‰) to ammonia. As the process involves very little isotopic fractionation, N_2-fixing plants grown on soils with little plant-available N are provided with a source of N with a $\delta^{15}N$ value close to 0‰ (Marshall et al., 2007; Virginia and Delwiche, 1982). In contrast, non-fixing plants rely solely on sources of N for which $\delta^{15}N$ values are determined by a variety of isotope fractionating processes. The resulting $\delta^{15}N$ values for non-fixing plants are not easy to predict, though it is thought that climate (rainfall, temperature), salinity and cultivation history may all have an effect (Amundson et al., 2003; Aranibar et al., 2008; Broadbent et al., 1980; Handley et al., 1999; Heaton, 1987; Kriszan et al., 2009). The $\delta^{15}N$ values of cereals (non-N_2-fixers) might be expected to show a more marked response to manuring than pulses (as N_2-fixers), with responses differing in different locations. It was therefore important to consider pulses, alongside cereals, because they form a large part of archaeobotanical seed-crop assemblages and probably make up a sizable component of human and potentially also animal diets.

2. Description of study sites

Table 1 provides a summary of the land-use history, climate, crop types, soil conditions and manuring regimes applied in each of the field experiments. Descriptions of the farm study locations are summarised in Table 2. Classification of the manuring regimes applied in the farm studies is based on our field observations and discussions with farmers.

2.1 Manuring at the long-term experimental sites

In the long-term agricultural experiments at Rothamsted (United Kingdom), Askov (Denmark) and Bad Lauchstädt (Germany) a variety of cereal crops was grown on well established unmanured and manured plots for over a century. They provide a unique opportunity to examine the response of cereal $\delta^{15}N$ values to soil manuring and non-manuring at a range of time scales. At all sites cattle were the source of animal manure. The rate of manure applied was different at each station. At Rothamsted and Askov, one level of manure was applied; Rothamsted manured plots received approximately 35 t/ha of cattle farmyard

manure (FYM 2) every year, while Askov manured plots (three replicates) received 37 t/ha of cattle slurry (SLU 1½) as an annual average over the four course rotation. At Bad Lauchstädt there were two levels of manure applied to separate plots every other year: the first plot received approximately 20 t/ha (FYM 1), the second plot received approximately 30 t/ha (FYM 2).

At Rothamsted, samples were obtained from two 'classical' experiments — Broadbalk Winter Wheat and Hoosfield Spring Barley — both of which began in the mid 1800s (Rothamsted Research, 2006). In addition to long-term manured and unmanured plots, the Hoosfield Spring Barley experiment also provided samples from a treatment that was manured until 1871 and thereafter unmanured (FYMr). Broadbalk winter wheat grain and rachis $\delta^{15}N$ values, a time series from 1852 to 2004, are published in Bogaard et al. (2007). At Askov, winter-sown bread wheat (*Triticum aestivum* L.) and spring-sown hulled two-row barley (*Hordeum vulgare* var. *distichum* L.) form a normal part of the crop rotation and were sampled in 2007; for this study, spring-sown naked two-row barley (*H. vulgare* var. *nudum*), spelt wheat (*Triticum spelta* L.) and emmer wheat (*Triticum dicoccum* Schrank) were also grown in 2008. At Bad Lauchstädt, bread wheat was sampled in 2001, 2002, 2004 and 2007 and spring barley was sampled in 2007 and 2008; the data from 2001-2 and 2004 are published in Bogaard et al. (2007).

2.2 Manuring at the short-term experimental sites

Two short-term field experiments were established to study the effects of manuring levels on crop $\delta^{15}N$ values after only one year of manure application. At Sutton Bonington, United Kingdom, the experiments were established on organically managed land, at SB-Site A in 2007 and SB-Site B in 2008. These were adjacent fields of similar soil type. The land entered organic conversion in 1999 (receiving no agrochemicals, fertilisers or manure since that time), though the previous history is unknown. At SB-Site A (2007), einkorn (*Triticum monococcum* L.), emmer and spelt wheat were grown on unmanured and manured plots, the manured soil receiving 35 t/ha of cattle manure (FYM 2). At the adjacent SB-Site B (2008), the experiment was repeated, with added replication (4 replicate plots per manure treatment and grain type, with 3 replicates in 2007) and an additional 'intermediate' manure level of 15-20 t/ha (FYM 1). Both fields underwent the same rotation scheme (Table 1) and both were preceded by leys, which were cut and mulched to return organic matter to the soil.

The second short-term field experiment was located 17 km north of Aleppo, Syria and established in a pistachio grove that had not received agrochemicals or manure in recent years. Bread wheat was grown without manure, with a 'medium' level (20 t/ha, FYM 1) and with a 'high' level (35 t/ha, FYM 2) of sheep manure. Irrigation was also incorporated in this experiment: all FYM 2 plots were also flood-irrigated six times during the growing season, while FYM 1 and unmanured plots were flood-irrigated either six or three times, or were not irrigated. The bread wheat experiment was repeated on different adjacent plots of land, AL-Site A in 2008 and AL-Site B in 2009. Precipitation differed in these two years: 2008 was a 'drought' year, while 2009 rainfall was closer to the regional average (Table 1).

2.3. Experimental pulses

A variety of pulse types from long- and short-term experimental sites were included in the study. At Bad Lauchstädt in 2007 and 2008, peas (*Pisum sativum* L.) and broad beans (*Vicia faba* L.) were grown in the same unmanured and manured plots as the cereals. In 2008 the pulses were grown in containers using soils from the fields to avoid rodents, which damaged the field-grown pulses the previous year. At Aleppo, lentils (*Lens culinaris* Medik.) were grown in separate plots under the same manure and irrigation treatments as the cereals. Lentil yields were low during 2008 due to low rainfall and, although pods formed, seed development was poor.

2.4. Farm studies

Three regions of 'traditional' farming – the Lena district of Asturias (Spain), the region east of Sighisoara in Transylvania (Romania) and the Aliveri district of Evvia (Greece) – were surveyed and sampled to assess the impact of manuring on $\delta^{15}N$ values in cereals and/or pulses in actual farm situations across different climatic zones (Figure 1, Table 2). In contrast with the field experiments, the farm manuring practices in a given year are likely to vary from one plot to the next, depending variously on proximity to animal stalling areas, the availability of manure (and thus the number and type of animals kept, degree of stall-feeding), means of transport and availability of human labour. With a view to archaeological interpretation of $\delta^{15}N$ crop values, the farm studies complement the field experiments by

providing data on the isotopic variability to be expected among plots within a village community or cluster.

Within these areas, only arable fields with no recorded use of chemical fertilizers were sampled. In two of the regions, Asturias and Sighisoara, pulses growing together with cereals in the fields were also collected: broad beans and peas in Asturias, lentils in Sighisoara. In the third region, central Evvia, farmers practised 'pure' pulse cultivation, mostly of broad beans (Table 2). Manuring intensity varied within each region, from annual manuring, through biennial or triennial manuring (of potatoes or maize grown every two or three years in rotation with cereals/pulses) to lack of manuring in recent years or decades. In the Evvia study, contrasts in long-term manuring practice were accounted for by classifying cultivation plots as 'highly manured' if in close proximity to the farmers' houses and/or stalling areas or, in the case of plots relatively distant from habitation, with a history of use as vegetable gardens. Cattle manure was used in Asturias and Sighisoara, while the farmers in Evvia used sheep/goat dung. Manuring rates quantifiable as tonnes per hectare could not be established in the farm studies.

3. Materials and methods

To achieve an average representation of crop $\delta^{15}N$ values in each field or experimental plot, the following procedure was employed. First, 30 cereal or pulse plants were harvested along a central transect through each experimental plot or farm study field. Second, 10 cereal ears, or pulse pods, were randomly chosen from the group of 30 plants. The grains from one side of each of the 10 cereal ears, or half the contents of the pulse pods, were removed for final sampling; all of the rachis (with attached glumes, in the case of the glume wheats einkorn, emmer and spelt) from the 10 cereal ears was also taken for analysis. Therefore, each bulk sample represents a pooled sub-sample from 10 different plants from each plot or field (for cereals ~200-300 grains and for pulses ~30-50 seeds). For cereals, the grain and rachis in these sub-samples were separated from each other and analysed separately. All crop samples were ground to a fine homogeneous powder (<250 μm size) under liquid nitrogen using a Spex 6850 freezer mill.

N contents were determined in a Costech 4010 elemental analyser (Costech, Florence) with %N calculated by comparison with standards calibrated against acetanilide. $^{15}N/^{14}N$ analysis was performed by sample combustion in a Flash 112 elemental analyser (ThermoQuest, Milan) linked under continuous flow with a Delta+XL mass spectrometer (ThermoFinnigan, Bremen). Isotope ratios were calculated as $\delta^{15}N$ versus atmospheric N_2 by comparison with standards calibrated against IAEA-N-1 (assumed $\delta^{15}N = +0.4‰$) and N-2 (assumed $\delta^{15}N = +20.3‰$), where:

$$\delta^{15}N = \left(\frac{{}^{15}N/{}^{14}N_{sample}}{{}^{15}N/{}^{14}N_{atmosN\,2}} - 1 \right) \times 10^{3}$$

The precision (1σ) among replicates of a homogenized barley sample was 0.2 for %N and 0.4‰ for $\delta^{15}N$ analysed in 29 separate runs.

Statistical analyses (using SPSS™ PASW Statistics version 18) were undertaken on the short- and long-term experimental sites to test the significance of the manuring treatments on pulse, grain and rachis $\delta^{15}N$ and %N values. The combined effects of manuring and irrigation were also assessed for the Aleppo data. The following tests were applied: an independent-sample t-test to the Rothamsted data, a two-way ANOVA with crop species and manure treatments as separate factors to the Sutton Bonington, Askov and Bad Lauchstädt data, and a two-way ANOVA with manure and irrigation levels as separate factors to the Aleppo data. For Bad Lauchstädt, sample sizes were too small for statistical analysis on separate years; therefore the data from all years were combined for pulses and cereals. A significance level of 0.05 was used for all statistical tests.

4. Results

4.1. Cereals grown in long-term field experiments

Tables 3a-c and Figure 2 summarise the results from the field experiments at Rothamsted (Broadbalk and Hoosfield), Bad Lauchstädt and Askov. Higher $\delta^{15}N$ values are associated with manured than with unmanured cereals in all three long-term experiments. These differences are visible across a range of species as well as in both grain and rachis. Overall,

the average offset between farmyard manured and unmanured grain $\delta^{15}N$ values was 5.5‰ at Rothamsted and 4.0‰ at Bad Lauchstädt. At Askov with manured plots taking cattle slurry, the difference between unmanured and manured plots averaged 9.0‰. Grain $\delta^{15}N$ values are consistently higher than rachis $\delta^{15}N$ values, a relationship that is explored further below.

The earliest archive samples available at Rothamsted indicate that a pronounced 'manuring effect' becomes apparent within a decade of plot establishment: by 1856 in the Hoosfield spring barley experiment (which began in 1852) $\delta^{15}N$ values are already offset by 2.5‰, and by 1852 in the Broadbalk wheat experiment (which began in 1844) $\delta^{15}N$ values are offset by 3.1‰. The Hoosfield Barley FYMr plot, which was manured from 1852 until 1871 (at the FYM2 level) only, records a gradual 3.5‰ decline in crop $\delta^{15}N$ values once manuring stopped over a 70-year period (until 1942). Between 1942 and 1962 there is an increase in $\delta^{15}N$ values across all treatments, which may reflect changes in harvesting and/or weed control techniques (Poulton, pers com.) The span of the archive samples analysed from Rothamsted indicates further that, while there is inter-annual variation through time, manured and unmanured plot values remain distinct. An overall decrease in unmanured crop $\delta^{15}N$ values through time is evident in both the Hoosfield and Broadbalk experiments, resulting in greater offsets between manured and unmanured plots at the end of both time series (7.6‰ and 5.8‰, respectively). Low crop $\delta^{15}N$ values in the unmanured plots probably reflect relatively small inputs of organic matter over the long term in comparison with the FYM plots.

While the results for bread wheat and hulled barley at Rothamsted, Bad Lauchstädt and Askov suggest similar manuring effects on both species, the additional data from Askov demonstrate that the effect of manuring is also similar for naked barley, emmer and spelt. The results indicate a remarkably consistent manuring effect across a broad range of cereals and confirm previous results from the Askov long-term experiments (Bol *et al*. 2005).

Different manuring levels have distinct impacts on the $\delta^{15}N$ values in cereals. At Bad Lauchstädt the higher of two manure application levels, FYM 2 (30t/ha biennially), is associated with ~1.5‰ higher crop $\delta^{15}N$ values than FYM 1 (20 t/ha biennially). More intensive manuring practices at Rothamsted (35 t/ha annually) and Askov (37 t/ha, annually) are associated with even higher $\delta^{15}N$ values. The highest manured cereal $\delta^{15}N$ values occur at Askov (8.5‰±0.3), where the use of cattle slurry with a relatively high ammonium content

(rather than farmyard manure with a larger fraction of the manure N in organic forms) may be a factor.

4.2. Cereals grown in short-term experiments

The manured and unmanured plots of einkorn, emmer and spelt wheat grown at Sutton Bonington provide data on cereal $\delta^{15}N$ values in the first cultivation season after cattle manure application. The results, summarised in Table 4a and Figure 3, demonstrate an appreciable but variable 'manuring effect' across the three cereal types in the two years. Unmanured plot $\delta^{15}N$ values at SB-Site A in 2007 are markedly higher than those at SB-Site B in 2008. The degree of $\delta^{15}N$ enrichment in the FYM 2 plots compared with unmanured plots also differs between the two years, with increases in 2007 averaging 2.6±0.8‰ versus an average difference of 1.0±0.4‰ in 2008. The magnitude of the manuring effect in this first year of manure application at Sutton Bonington is lower than that observed in the long-term experimental fields but comparable to the earliest archive samples at Rothamsted, when unmanured values were still relatively high. Overall, the Sutton Bonington results suggest that: 1) the degree of manuring impact on cereal $\delta^{15}N$ values can vary between sites and years; 2) the difference between manured and unmanured plots in the first season after initial application is smaller than the difference after long-term cultivation with/without manure; and 3) there are no obvious differences in manuring effect amongst the three hulled wheat species.

At Aleppo, Syria, where manuring was combined with irrigation, analysis of bread wheat grown at AL-Site A in 2008 on newly established plots registered a low-level 'manuring effect' of 1.9‰ (Table 4b, Figure 3). In 2009 at AL-Site B, however, $\delta^{15}N$ values of bread wheat grown on newly established plots, immediately adjacent to those cultivated in the previous year, exhibited a much more dramatic manuring impact, particularly where manuring was combined with full irrigation, resulting in an average offset of 3.5‰. These different offsets may be related to the marked contrast in annual rainfall received between 2007 and 2008; even though irrigation levels were the same for each year, rainfall was approximately 60 mm higher in 2009 (see Table 1). Overall, the Syrian results suggest that the effect of manuring on cereal $\delta^{15}N$ may also be dependent on water availability, whether derived from precipitation or from irrigation.

Combined with the analysis of early archive samples from Rothamsted (above), the results of the short-term experiments suggest that significant ^{15}N enrichment can emerge in the first cultivation season following initiation of intensive manuring, but much depends on site-specific conditions and, particularly in arid regions, on water availability.

4.3. Experimentally grown pulses

The $\delta^{15}N$ values of pulses grown at Bad Lauchstädt, under the same manured and unmanured soils as those for the cereals (including field- and container-grown pulses), are shown in Table 3c and are plotted in Figure 4. The results show only small or no enrichment of $\delta^{15}N$ values (mean +0.2‰) at the FYM1 level of manuring and a slightly larger effect (mean +0.7‰) at the highest FYM2 level of manuring. Given that the pulses were grown in cereal plots in 2007 but in pots in 2008, it is unlikely that root competition with cereals, which have more extensive rooting systems than pulses, can explain the low pulse values.

At Aleppo, Syria, lentils grown in 2008 and 2009 on newly established plots also showed only a slight tendency (mean +0.3‰) towards ^{15}N enrichment at the FYM1 level of manuring (both irrigated and non-irrigated). The higher FYM2 manured plots (here, combined with high irrigation) showed a larger response (mean +0.8‰) to manuring (Table 4b, Figure. 4). Yield data collected as part of the experiment in 2008 showed that the yield of manured lentil plots was greater (averaging 212 kg seed weight/dunum), than that of unmanured lentil plots (averaging 174 kg/dunum), therefore confirming that manuring was indeed beneficial despite its low impact on $\delta^{15}N$ values.

Overall, the low $\delta^{15}N$ values of the manured pulses compared to the manured cereals indicate that they are still heavily affected by fixation of atmospheric nitrogen, even when they are intensively manured at the levels applied in the experimental studies (i.e. range of 20 to 35 t/ha). Any tendency towards ^{15}N enrichment of pulses associated with low manure levels (here, < FYM1, ~15-20t/ha) maybe too slight to distinguish from overall analytical measurement precision (here, ±0.4‰).

4.4. Farm studies

Tables 5a-b and Figure 5 summarise the results from the farm studies. As in the datasets from the long- and short-term experiments, rachis values are consistently lower than grain values (see also below). Enrichment of cereal $\delta^{15}N$ values is evident amongst manured soils but is also variable. This variation is attributable at least in part to manuring intensity and/or history; for example, the highest spelt $\delta^{15}N$ value from Asturias (10.1‰) derived from an unusual plot in which the farmer had manured the spelt very intensively, "like potatoes".

Comparison of the two studies featuring combined cereal and pulse cultivation (Asturias and Sighisoara) shows that, in both cases, pulses always have lower values than cereals growing in the same plots. This observation is consistent with the ability of pulses to make greater use of fixed atmospheric N. While it could be argued that the deeper rooting systems of cereals in comparison with pulses enables them to compete successfully for mineral soil N, as noted above pulses grown experimentally in pots also show relatively low $\delta^{15}N$ values.

The highest $\delta^{15}N$ values of any pulses measured in the project derive from the third farming region, central Evvia, Greece (Table 5c, Figure 5). Farmers here manured some pulse plots very intensively, resulting in the formation of an artificial 'dung-soil' (*koprókhoma*). On average, highly manured pulses have higher $\delta^{15}N$ values than those grown on plots receiving low or infrequent manure application (Table 5c).

4.5. Results of statistical analyses and the relationships between $\delta^{15}N$ and %N in grain and rachis

The results of the statistical analyses conducted on separate sites and crop species are summarised in Tables 6 and 7. Overall, the effect of the manure treatments on experimental cereal grain $\delta^{15}N$ values is highly significant ($p<0.01$) for all sites with the exception of Sutton Bonington Site-B in 2008 ($p=0.313$). Cereal grain $\delta^{15}N$ values at Aleppo showed a larger response to manuring than to irrigation. The effect of irrigation on cereal grain $\delta^{15}N$ values at Aleppo was only significant in 2009 (note also the higher level of rainfall that year).

There was a significant effect of FYM2 manuring on lentil $\delta^{15}N$ values at Aleppo in both years; yet no significant effects were evident for irrigation. In contrast, manuring had no significant effect on pulse $\delta^{15}N$ values at Bad Lauchstädt in either year sampled, yet the

significant effect of species ($p < 0.05$) indicates that peas and broad beans responded slightly differently to manure application; peas had $\delta^{15}N$ values on average 0.3‰ higher (see Figure 4a) than broad beans. In light of these results, a two-way was ANOVA conducted on the sites where pulses and cereals were grown together (Aleppo and Bad Lauchstädt), which included crop type (i.e. cereal or pulse) and manure treatment as factors. The main effects of crop type and manuring on $\delta^{15}N$ values were both significant (Bad Lauchstädt: crop type $F(2,10)=153.2$, $p \leq .000$ and manure $F(2,10)=36.9$, $p \leq .000$. ; Aleppo: crop type $F(2,107)=45.5$, $p \leq .000$ and manure $F(2,107)=20.3$, $p \leq .000$). Furthermore, there was a significant interaction between crop type and manure treatment (Bad Lauchstädt: $F(2,10)=20.7$, $p \leq .000$. Aleppo: $F(2,107)=11.1$, $p \leq .000$), which statistically confirms overall that the pulses and cereals responded differently to the manure treatments.

Manuring had much less of an effect on grain %N values than on $\delta^{15}N$ values; the only significant effect of manure treatment (FYM 2) on %N was observed in Askov crops in 2007 (unmanured M (mean) =1.2, SD 0.0, FYM 2 M=1.4, SD 0.2, $p<0.05$). During 2007, there was also a small, yet only just significant, effect of cereal species on %N at Askov ($p =0.04$), where hulled two-row barley had marginally higher %N than bread wheat (M=1.3, SD 0.1, and M=1.5, SE 0.1, respectively). A significant effect of cereal species on %N was also observed at Sutton Bonington SB-Site A in 2007, where spelt had lower %N (M=1.8, SD=0.2) than emmer and einkorn wheat (M=2.1, SD=0.3 and M=2.5, SD=0.3, respectively).

Statistical analysis of the cereal rachis $\delta^{15}N$ and %N values (Table 7) produced very similar results to that of the cereal grain. The main difference is that manuring had no significant impact on rachis $\delta^{15}N$ values at Aleppo AL-Site B in 2009.

The study observed that cereal rachis $\delta^{15}N$ values were consistently lower than cereal grain $\delta^{15}N$ values across all of the study sites, and this relationship is shown in Figure 6. The mean offset between grain and rachis was 2.4 ±0.8‰ (Pearson product-moment correlation coefficient: $r=0.962$, $n=179$, $P<0.001$). Nevertheless, rachis $\delta^{15}N$ values show the same magnitude of manuring effect as cereal grain. Rachis also had lower %N values than cereal grains and pulses: mean %N was 0.3% ±0.2 for all rachis samples, 1.6% ±0.3 for all cereal grain samples and 3.9% ±0.2 for all pulse samples.

5. Geographical variation

The experimental sites in this study were spread across a wide geographic area with a range of markedly different climatic conditions and soil types (see Table 1 and Figure 1). Previous studies have observed that relatively warm, dry climates are associated with higher plant $\delta^{15}N$ values than cooler and wetter temperate ones (e.g. Amundson et al. 2003; Aranibar et al. 2004; Handley et al. 1999a; Heaton 1987). In order to investigate this specific plant/climate association in relation to our crop study, Pearson product-moment correlation coefficients were computed to assess relationships between experimental cereal $\delta^{15}N$ values (unmanured and FYM 2) and site-specific mean annual precipitation (MAP). There was no significant correlation between unmanured cereal $\delta^{15}N$ values and MAP, r=-0.304, n=13, p=0.313. However, there was a significant positive correlation between FYM 2 manured cereal $\delta^{15}N$ values and MAP, r=0.984, n=13, p=0.000. Scatter plots summarize these results (see Figure 7a and 7b). It therefore appears that generalized geographical trends towards ^{15}N enrichment with decreasing rainfall are not apparent in the unmanured cereals sampled in this project, and that the opposite trend—towards higher $\delta^{15}N$ values with increasing rainfall—is evident among the intensively manured FYM2 treatments. In considering these geographical patterns, however, it may not be realistic to compare broad global trends of entire ecosystems (Amundson et al., 2003; Handley et al., 1999a) with the restricted range of agricultural conditions under which our crops were grown. In addition it must be accepted that the relationship between plant $\delta^{15}N$ values and water availability is a complex issue, with conflicting results (both positive and negative relationships) from different studies (Amundson et al. 2003; Lopes and Araus, 2006; Yousfi et al., 2010). Moreover, the positive FYM2 vs. MAP trend (Figure 7b) could be more apparent than real when duration of the manure regimes are considered: low values at Aleppo may reflect long-term cultivation without manuring prior to establishing the short-term experiment (only one year of manure application so far); the FYM2 treatment at Bad-Lauchstädt is applied *biennially*; and lower values at Sutton Bonington than at Rothamsted may reflect the short-term nature (also only one year of manure application so far) of intensive manuring at the former.

6. Discussion

The experimental results synthesized here suggest clear causal links between cereal $\delta^{15}N$ values and the intensity and longevity of cultivation with and without manuring. Contrasting

levels and frequencies of application amongst the long-term experiments (Table 3) are reflected in differing offsets between manured and unmanured treatments (Figure 2). Though subtle variation in manuring impact on different cereal species cannot be excluded, it is clear that different cereal species grown under the same conditions have similar values.

The long-term field experiments suggest that a baseline range of $\delta^{15}N$ values can be established to interpret archaeobotanical cereal $\delta^{15}N$ values (see Figure 2). The time-series available from the Rothamsted archives, as well as Askov and Bad Lauchstädt, indicate that cereals grown without manuring over the long term are low in ^{15}N, resulting in values below ~2.5‰. By contrast, long-term continued cultivation of cereals with high levels of annual manuring (~35-37 t/ha, applied annually), as at Rothamsted and Askov, results in high values of ~6‰ or higher.

Medium values between ~2.5‰ and ~6‰ could reflect several scenarios: lower levels of manure application over the long-term (as in the biennial application of ~20-30 tons/a at Bad Lauchstädt); the residual effects after a period of intensive manuring (as in the FYMr plot at Rothamsted), or the early years of a new cultivation regime (as in the early archive samples at Rothamsted). The latter scenario could be excluded archaeobotanically if associated arable weed assemblages suggest long established cultivation plots (Bogaard 2002).). Applying these baselines archaeologically would require not only complementary information from available weed evidence but also relevant palaeoecological contextualisation with regard to other possible causes of ^{15}N enrichment (e.g. wetland denitrification, salinity etc.).

While long-term manuring practices are sufficiently marked that they might be discernible archaeologically, the results of the short-term field experiments suggest that crops grown after an initial season of intensive manure input will not necessarily register a marked effect on $\delta^{15}N$ values. Though these results limit the resolution of land-use inferences as regards short-term manuring, they also suggest that crop $\delta^{15}N$ values indicative of high manuring levels reflect long-term continuity, with consequent implications for the stability of land tenure. The 'slow-release' nature of manuring effects on soils (DEFRA, 2010) suggests that the practice lends itself to long-term investment in arable land.

The results presented also confirm previous indications based on pilot work (Bogaard et al. 2007) of a systematic offset between cereal grain and rachis. This consistent result (Figure 6)

has significant implications both for the interpretation of archaeobotanical grain versus chaff values and also for palaeodietary work, where the values of plants consumed by humans and other animals are often assumed to be similar. If cereal grain were mostly consumed by humans and rachis/glumes by animals, it is evident that the offset between these plant sources would approximate the trophic level effect. Work on other plant parts suggests that straw, leaves and other structural plant parts are similarly low in $\delta^{15}N$ relative to grain (Smolinska unpublished data 1991 cited in Bogaard et al. 2007). The offset in $\delta^{15}N$ values between grain and other plant parts suggests that it may be possible to distinguish between animal foddering with chaff and with grain, which have markedly different implications for both animal productivity and cost to the farmer.

The effect of manuring on pulses is comparatively slight, possibly due to the pervasive impact of atmospheric N_2-fixation, especially when grown on soils with very little mineral N (ammonium and nitrate) availability. In one study, Evvia in Greece, where broad beans and other pulses were in some cases treated with extremely high levels of manuring over an extended period, certain manured pulse values were significantly offset from unmanured values (Figure 5c). It is likely that the highly manured soils in Evvia have abundant mineral N, which results in pulses taking up mineral soil N and reducing their N_2-fixing activity, and thereby achieving $\delta^{15}N$ values that overlap with the signatures of manured cereal values observed in other farming studies. The implication is that very intensive manuring of pulses would be detectable archaeobotanically, whereas lower levels would not. In palaeodietary terms, it appears that pulses would generally contribute low $\delta^{15}N$ values ($\leq$2.5‰), unless treated with extremely high levels of manure.

Finally, the comparison of experimental and 'real' farming studies is important for framing potential interpretation of archaeobotanical crop $\delta^{15}N$ values. The farm studies register considerable variation amongst plots forming part of the same general manuring regime. This apparent variability in actual manuring level probably reflects a combination of factors, including scarcity of manure, resulting in selective use, as well as variation amongst farming families in available time/labour for spreading manure.

The observation that fairly consistent values for cereals and pulses occur across the studies is significant for archaeological inferences on both land use and palaeodiet. For land use reconstruction, it appears that manuring has similar enrichment effects in different climate

zones and soil types. The finding that the unmanured cereal grain $\delta^{15}N$ values observed in this study do not follow the commonly observed negative relationship with local mean annual rainfall (Figure 7a) indicates that climatic conditions, here represented by a single meteorological variable such as rainfall, are not the dominant determining factor. Cereal $\delta^{15}N$ values, especially at the long-term experimental sites, are predominately responding to the application of high levels of manure and soils with a readily available source of ^{15}N enriched mineral N, more so than to precipitation or soil moisture balance levels in isolation. Long-term intensive manuring of arable soils increases the overall N content and microbial action, which leads to greater decomposition and mineralization of organic N and further increases the likelihood of conditions that result in ^{14}N losses, mobilised either by volatilisation or leaching. These losses may increase with rainfall/water availability thus enhancing the manuring effect (Figure 7b), but the lack of long-term intensive annual manuring in medium to low-level rainfall areas amongst our sites restricts our ability to explore this relationship. Nevertheless, it seems clear that crop $\delta^{15}N$ values in this study derive from a range of artificially 'buffered' growing conditions and do not reflect the plant/climate trends observed in natural habitats elsewhere (e.g., Amundson et al. 2003; Heaton 1987).

In terms of palaeodiet, interpreting high $\delta^{15}N$ values in *crops* as indicative of aridity would be problematic given the lack of correlation between unmanured cereal $\delta^{15}N$ and rainfall and the observed 'enriching' effects that land use practices like manuring can have on crop $\delta^{15}N$ values in a range of high and low-rainfall areas. Although the effects of aridity on a broad range of plant $\delta^{15}N$ values are observed at a variety of local and global scales (e.g., Handley et al. 1999b; Hartman and Danin 2009; Heaton 1987; Schwarcz et al. 1999), this study suggests that other explanations for elevated $\delta^{15}N$ values in human or animal consumers should be considered. For palaeodiet interpretations, this inference would ideally be assessed directly through isotopic analysis of archaeobotanical remains.

7. Conclusion

Our data suggest that long-term cultivation with or without manuring can result in radically different cereal $\delta^{15}N$ values; middling values are associated with the early years of a manuring regime or with moderate manure levels. Moreover, $\delta^{15}N$ values in different cereal plant parts (specifically, grain and rachis) differ systematically. Unless very intensive over an extended period, the impact of manuring on $\delta^{15}N$ values in pulses is slight or lacking

altogether. Measurement of $\delta^{15}N$ values in archaeobotanical remains is desirable both as direct evidence of crop growing conditions and crop husbandry practices and as a central component of palaeodietary investigations.

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Table 1

Summary of the experimental field sites

Table 2

Summary of the farming study locations

Table 3a

Results for the analysis of bulk samples from, Rothamsted, UK, A) Hoosfield Barley Experiment, and B) Broadbalk Winter Wheat Experiment (data from Bogaard et al., 2007 are included for comparison): Nil = unmanured; FYM2= 35t/ha manure; FYMr- manured from 1852 until 1871and unmanured thereafter; na = material not available for analysis (no replicate plots per treatment).

Table 3b

Results for the analysis of bulk samples from Askov, Denmark: Nil = unmanured plot; SLU 1½ = 35t/ha manure slurry (average of three replicate plots per treatment, with 1 SD in parentheses).

Table 3c

Results for the analysis of bulk samples from Bad Lauchstädt, Germany: Nil = unmanured plot; FYM1 = 20t/ha manure; FYM2 = 35t/ha manure; na = material not available for analysis (no replicate plots per treatment).

Table 4a

Results for the analysis of bulk samples from Sutton Bonington, United Kingdom: Nil = unmanured plot; FYM 1 = 15-20t/ha manure; FYM 2 = 35t/ha manure; na= not available (average of three replicate plots per treatment, with 1 SD in parentheses).

Table 4b

Results for the analysis of bulk samples from Aleppo, Syria: Nil-a = no manure/no irrigation, Nil-b = no manure/medium irrigation; Nil-c = no manure/full irrigation; FYM 1a = medium (20t/ha) manure/no irrigation; FYM1-b = medium (20t/ha) manure/medium irrigation; FYM1-c = medium (20t/ha) manure/full irrigation; FYM2 = high (35t/ha) manure/full irrigation (average of three replicate plots per treatment, with 1 SD in parentheses).

Table 5a

Results for the analysis of bulk samples from the Lena district, central Asturias, Spain: Na = material not available for analysis; nd = not determined; Not Recent = no recent manure applied; Biennial = manure applied every second year; Direct = manure applied during sampled growing season (an average of 3 to 4 replicate fields were sampled per manure level (when available), with 1 SD in parentheses)

Table 5b

Results for the analysis of bulk samples from the Sighisoara region, Transylvania, Romania: na= material not available for analysis; nd= not determined; Not recent = no recent manure applied; Triennial = manure applied every third year; Direct = manure applied during sampled growing season (an average of 3 to 4 replicate fields were sampled per manure level (when available), with 1 SD in parentheses).

Table 5c

Results for the analysis of bulk samples from the Aliveri district of central Evvia, Greece: Low = low manure, High= high manure (an average of 3 to 4 replicate fields were sampled per manure level (when available), with 1 SD in parentheses).

Table 6

Results of two-way ANOVA tests (t-test applied to Rothamsted) on cereal grain and pulse, A) $\delta^{15}N$ values and, B) %N values (F(df effect, df error) = F value, p-value. † indicates data from all years were combined because sample sizes were too small for statistical analysis on separate years, na = not applicable, * significant response at an alpha level of 0.05).

Table 7

Results of two-way ANOVA tests (t-test applied to Rothamsted) on cereal rachis, A) δ15N values and, B) %N values (F(df effect, df error) = F value, p-value. † indicates data from all years were combined because sample sizes were too small for statistical analysis on separate years, na = not applicable, * significant response at an alpha level of 0.05).

Table 1

Summary of the experimental field sites

Experiment	Location	Period	Climate (Köppen-Geiger)	Soil type	Annual rainfall (mm)	Cropping regime	Crop(s) sampled	Manuring treatment(s)	Plot(s) sampled	References
Long-term:										
Broadbalk Winter Wheat, Rothamsted	Hertfordshire, United Kingdom	1844-present	Temperate oceanic	Chromic luvisol; flinty-silty clay loam over Clay-with-flints	727	Autumn-sown bread wheat	*Triticum aestivum*	Cattle manure, 35 tonnes/ha every year	Single plot per treatment	Rothamsted Research 2006
Hoosfield Spring Barley, Rothamsted	Hertfordshire, United Kingdom	1852-present	Temperate oceanic	Chromic luvisol; flinty-silty clay loam over Clay-with-flints	727	Spring-sown hulled two-row barley	*Hordeum vulgare* var. *Distichum*	Cattle manure, 35 tonnes/ha every year	Single plot per treatment	Rothamsted Research 2006
Askov Long-Term Experiment	South Jutland, Denmark	1894-present	Temperate oceanic/cool temperate	Alfisol (Typical Hapludalf); coarse sandy loam	862	Autumn-sown bread wheat/root crops/spring hulled two-row barley/clover and grass (4-course rotation)	*Triticum aestivum*; *Hordeum vulgare var. distichum*; *Triticum dicoccum*; *Triticum spelta*; *Hordeum vulgare* var. *nudum**	Cattle slurry, *c.* 37 tonnes/ha (mean across rotation)	Three replicate plots per treatment	Christensen et al. 2006
Static Fertilization Experiment, Bad Lauchstädt	Leipzig-Halle, Germany	1902-present	Cool temperate	Haplic chernozem; loess	483	Autumn-sown bread wheat/sugar beet/spring-sown hulled two-row barley/potatoes (4-course rotation)	*Triticum aestivum*; *Hordeum vulgare var. distichum; Lens culinaris*; *Pisum sativum*; *Vicia faba***	Cattle manure, 20 and 30 tonnes/ha every second year	Single plot per treatment	Körschens and Pfefferkorn 1998
Short-term:										
Sutton Bonington	Nottinghamshire, United Kingdom	2007-8	Temperate oceanic	Stagnogleic argillic brown earth	606	Autumn-sown einkorn, emmer and spelt wheat/winter beans/winter oat/clover or vetch and ryegrass (4-course rotation)	*Triticum monococcum*; *Triticum dicoccum*; *Triticum spelta*	Cattle manure, 35 tonnes/ha, plus intermediate level of 15-20 tonnes/ha in 2008	Four replicate plots per treatment, randomised layout	
Aleppo	Syria	2008-9	Warm Mediterranean	Light brown chalky soil	201 mm (2008), 263 mm (2009)	Autumn-sown bread wheat and winter-sown lentils	*Triticum aestivum*; *Lens culinaris*	Sheep manure, 20 and 30 tonnes/ha on wheat, 10 and 20 tonnes/ha on lentils	Four replicate plots per treatment, randomised layout	

*in 2008, spring-sown emmer, spelt and naked two-row barley were grown on manured and control plots

**in 2007, spring-sown pea, lentil and broad bean were grown in small plots in the established FYM1-2 and control treatment areas; in 2007, these pulses were grown under equivalent conditions in pots

Table 2

Summary of the farming study locations

Region	**Climate (Köppen-Geiger)**	**Soil type(s)**	**Annual rainfall (mm)**	**Cropping regime(s)**	**Crop(s) sampled**	**Manuring regime(s)**
Lena district, central Asturias, Spain	Temperate oceanic	range from light to heavy (clay-rich)	c. 1000-1100	Autumn/winter-sown spelt or emmer/spelt maslin (sometimes with peas or broad beans) usually rotated with potatoes and/or maize, occasionally with other garden crops; rarely continuous spelt for several years	*Triticum spelta*; *Triticum dicoccum*; *Vicia faba*; *Pisum sativum*	Normally biennial, with cattle manure applied to potatoes or maize in year prior to sampling; occasionally direct manuring of sampled spelt (otherwise avoided due to problems of lodging); occasionally no manure for several years
Sighisoara region, Transylvania, Romania	Temperate continental	pale brown sandy loam	c. 650	Autumn- and spring-sown einkorn, spelt, barley, bread wheat, sometimes with pulses (bitter vetch and lentil)	*Triticum monococcum*; *Triticum aestivum*; *Lens culinaris*	Variable, ranging from no manure in recent years to direct manuring of cereals in the sampling year
Aliveri district, central Evvia, Greece	Warm-mediterranean	red soil [*kokkinókhoma*], white soil [*asprókhoma*], brown soil [*melissókhoma*], 'dung soil' [*koprókhoma*]	c. 430	Winter-sown pulses (mostly broad bean) grown continuously or rotated with summer vegetables, potatoes, cereals (oats, barley) for hay	*Vicia faba*; *Pisum sativum*; *Lathyrus ochrus* (fava)	Variable, ranging from intensive annual manuring with sheep/goat dung to intermittent manuring (including by grazing or penned sheep/goats) to no manuring in recent years or decades

Table 3a

Results for the analysis of bulk samples from, Rothamsted, UK: A) Hoosfield Barley Experiment, and B) Broadbalk Winter Wheat Experiment (data from Bogaard et al., 2007 are included for comparison). Nil = unmanured; FYM2= 35t/ha manure; FYMr- manured from 1852 until 1871and unmanured thereafter; na = material not available for analysis (no replicate plots per treatment).

A. Hoosfield Barley

Year	$\delta^{15}N$						%N					
	Grain			Rachis			Grain			Rachis		
	Nil	FYM2	FYMr	Nil	FYM2	FYMr	Nil	FYM2	FYMr	Nil	FYM2	FYMr
1856	2.8	5.3	na	na	na	na	1.6	1.6	na	na	na	na
1871	2.8	6.5	na	na	na	na	1.3	1.7	na	na	na	na
1882	2.8	8.6	6.0	-1.5	4.5	1.7	1.2	1.6	1.3	0.4	0.4	0.4
1902	2.5	6.8	4.3	-0.3	2.8	2.1	1.4	1.9	1.7	0.3	0.4	0.4
1923	2.1	8.6	3.1	na	na	1.4	1.7	1.8	1.2	na	na	0.7
1942	1.2	8.2	2.5	-1.4	3.7	1.6	1.3	1.6	1.3	0.4	0.4	0.5
1962	1.6	9.2	4.4	-0.4	6.6	2.5	1.4	1.9	1.5	0.5	0.9	0.7

B. Broadbalk Winter Bread wheat

Year	$\delta^{15}N$				%N			
	Grain		Rachis		Grain		Rachis	
	Nil	FYM2	Nil	FYM2	Nil	FYM2	Nil	FYM2
1852	2.7	5.8	na	na	1.8	2.0	na	na
1895	2.9	7.8	0.6	5.0	1.9	1.8	nd	nd
1935	4.0	8.3	0.4	7.1	1.8	nd	0.6	0.6
1965	0.7	7.4	-2.5	5.3	2.0	2.3	0.3	0.8
1991	0.6	8.6	na	na	1.5	2.0	na	na
2004	0.8	6.6	-2.6	3.4	1.5	1.9	nd	nd

Table 3b

Results for the analysis of bulk samples from Askov, Denmark: Nil = unmanured plot; SLU 1½ = 35t/ha manure slurry (average of three replicate plots per treatment, with 1 SD in parentheses).

Year	$\delta^{15}N$							
	Grain				Rachis			
	Nil		SLU 1½		Nil		SLU 1½	
Bread wheat								
2007	-2.4	(0.6)	8.1	(1.3)	-4.1	(0.6)	5.3	(0.7)
Hulled two-row barley								
2007	-0.1	(0.6)	8.7	(1.9)	-2.1	(0.4)	5.7	(2.4)
Emmer								
2008	-0.6	(0.2)	8.9	(0.6)	-2.1	(0.5)	5.9	(0.4)
Spelt								
2008	-0.1	(0.2)	8.2	(2.0)	-1.9	(0.2)	6.3	(1.8)
Naked two-row barley								
2008	0.2	(0.5)	8.4	(1.3)	-2.2	(0.7)	5.3	(1)

Year	%N							
	Grain				Rachis			
	Nil		SLU 1½		Nil		SLU 1½	
Bread wheat								
2007	1.2	(0.1)	1.3	(0.1)	0.2	(0)	0.2	(0)
Hulled two-row barley								
2007	1.2	(0)	1.5	(0.1)	0.3	(0.1)	0.4	(0.2)
Emmer								
2008	1.9	(0)	1.8	(0.1)	0.2	(0)	0.2	(0)
Spelt								
2008	2.1	(0.1)	2.1	(0.1)	0.3	(0.1)	0.3	(0)
Naked two-row barley								
2008	1.8	(0.1)	1.8	(0.2)	0.4	(0)	0.4	(0)

Table 3c

Results for the analysis of bulk samples from Bad Lauchstädt, Germany: Nil = unmanured plot; FYM1 = 20t/ha manure; FYM2 = 35t/ha manure; na = material not available for analysis (no replicate plots per treatment).

Year	$\delta^{15}N$						%N					
	Grain or pulse			Rachis			Grain or pulse			Rachis		
	Nil	FYM1	FYM2	Nil	FYM1	FYM2	Nil	FYM1	FYM2	Nil	FYM1	FYM2
Bread wheat												
2001	1.0	4.1	5.7	na	na	na	1.2	1.1	1.3	na	na	na
2002	0.1	2.7	4.1	na	na	na	1.4	1.4	1.5	na	na	na
2004	0.8	3.2	3.4	-1.3	0.9	2.2	1.1	1.3	1.3	0.2	0.3	0.3
2007	0.0	3.2	5.0	-2.2	1.2	3.3	1.4	1.7	1.8	0.5	0.6	0.7
2008	1.9	4.1	4.9	0.0	2.0	3.0	1.1	1.2	1.3	0.2	0.3	0.3
Hulled two-row barley												
2007	-0.3	3.3	3.9	-2.8	0.6	1.5	1.5	1.6	1.5	0.5	0.4	0.3
2008	-0.3	3.4	4.3	-1.8	1.8	3.1	1.1	1.3	1.3	0.2	0.3	0.4
Pea												
2007	-0.3	0.3	0.5				3.4	3.7	3.7			
2008	0.0	0.2	0.8				3.0	3.0	3.0			
Broad bean												
2007	-0.3	0.0	0.2				4.2	5.1	4.6			
2008	-0.6	-1.0	0.0				4.1	4.3	3.7			

Table 4a

Results for the analysis of bulk samples from Sutton Bonington, United Kingdom: Nil = unmanured plot; FYM 1 = 15-20t/ha manure; FYM 2 = 35t/ha manure; na= not available (average of three replicate plots per treatment, with 1 SD in parentheses).

Year	$\delta^{15}N$											
	Grain						Rachis					
	Nil		FYM 1		FYM 2		Nil		FYM 1		FYM 2	
Emmer												
Site A, 2007	4.8	(0.9)	na		6.9	(0.1)	2.7	(1.8)	na		4.0	(1.5)
Site B, 2008	3.2	(2.2)	3.0	(1.0)	3.8	(1.9)	0.6	(2.0)	0.5	(1.3)	1.3	(1.8)
Einkorn												
Site A, 2007	5.4	(0.8)	na		7.7	(0.1)	2.9	(1.2)	na		4.8	(0.6)
Site B, 2008	1.8	(1.3)	2.6	(1.3)	3.2	(0.1)	-0.7	(1.4)	-0.1	(1.2)	0.7	(0.9)
Spelt												
Site A, 2007	4.6	(0.7)	na		8.1	(2.5)	1.0	(0.9)	na		3.9	(2.7)
Site B, 2008	2.5	(2.1)	3.3	(0.6)	3.5	(1.8)	0.2	(1.4)	0.5	(1.0)	0.9	(1.6)

Year	%N											
	Grain						Rachis					
	Nil		FYM 1		FYM 2		Nil		FYM 1		FYM 2	
Emmer												
Site A, 2007	1.8	(0.7)	na		2.1	(0.3)	0.4	(0)	na		0.4	(0.1)
Site B, 2008	1.8	(0.3)	1.9	(0.1)	1.9	(0.1)	0.2	(0)	0.2	(0)	0.2	(0.1)
Einkorn												
Site A, 2007	2.2	(0.2)	na		2.5	(0.3)	0.5	(0.1)	na		0.5	(0.1)
Site B, 2008	1.9	(0.1)	1.9	(0.1)	2.2	(0.3)	0.2	(0.1)	0.2	(0)	0.2	(0)
Spelt												
Site A, 2007	1.7	(0.1)	na		1.9	(0.2)	0.2	(0)	na		0.3	(0.1)
Site B, 2008	1.9	(0.3)	1.9	(0.2)	1.9	(0.2)	0.2	(0)	0.2	(0)	0.2	(0)

Table 4b

Results for the analysis of bulk samples from Aleppo, Syria: Nil-a = no manure/no irrigation, Nil-b = no manure/medium irrigation; Nil-c = no manure/full irrigation; FYM1-a = medium (20t/ha) manure/no irrigation; FYM1-b = medium (20t/ha) manure/medium irrigation; FYM1-c = medium (20t/ha) manure/full irrigation; FYM2 = high (35t/ha) manure/full irrigation (average of three replicate plots per treatment, with 1 SD in parentheses).

Year	$\delta^{15}N$													
	Grain													
	Nil-a		Nil- b		Nil-c		FYM 1- a		FYM 1- b		FYM 1- c		FYM 2	
Bread wheat														
Site A, 2008	-0.7	(0.2)	-0.5	(0.3)	0.1	(0.4)	1.4	(0.6)	0.5	(0.6)	0.9	(0.2)	1.9	(0.4)
Site B, 2009	0.6	(0.6)	0.0	(0.5)	1.3	(1.1)	4.5	(0.7)	3.7	(0.4)	6.0	(0.6)	4.8	(0.7)
Lentil														
Site A, 2008	-0.6	(0.2)	0.1	(0.4)	0.1	(0.2)	0.0	(0.3)	0.0	(0.2)	0.0	(0.5)	0.4	(0)
Site B, 2009	-0.3	(0.3)	-0.5	(0.2)	-0.1	(0.2)	-0.3	(0.2)	0.3	(0.4)	0.3	(0.2)	0.7	(0.4)

Year	$\delta^{15}N$													
	Rachis													
	Nil-a		Nil- b		Nil-c		FYM 1- a		FYM 1- b		FYM 1- c		FYM 2	
Bread wheat														
Site A, 2008	-2.7	(0.4)	-2.6	(0.2)	-2.0	(0.9)	-1.1	(0.8)	-2.2	(0.9)	-1.3	(0.3)	-0.7	(0.5)
Site B, 2009	-0.5	(0.9)	-0.9	(0.4)	-0.7	(0.5)	3.3	(0.9)	3.3	(1.5)	4.7	(2.3)	3.5	(0.5)

Year	%N													
	Grain													
	Nil-a		Nil- b		Nil-c		FYM 1- a		FYM 1- b		FYM 1- c		FYM 2	
Bread wheat														
Site A, 2008	1.3	(0.1)	1.2	(0.2)	1.8	(0.7)	1.3	(0.1)	1.4	(0.2)	1.3	(0)	1.4	(0.1)
Site B, 2009	1.4	(0.1)	1.4	(0.1)	2.1	(0.7)	1.4	(0)	1.5	(0.2)	1.4	(0.1)	1.6	(0.1)
Lentil														
Site A, 2008	4.0	(0.1)	3.8	(0.2)	4.1	(0.3)	3.7	(0.2)	4.0	(0.2)	3.9	(0.1)	3.9	(0.1)
Site B, 2009	3.5	(0.1)	3.8	(0.2)	3.8	(0.5)	3.6	(0.1)	3.9	(0.2)	3.6	(0.4)	3.7	(0.1)

Year	%N													
	Rachis													
	Nil-a		Nil- b		Nil-c		FYM 1- a		FYM 1- b		FYM 1- c		FYM 2	
Bread wheat														
Site A, 2008	0.3	(0.1)	0.3	(0.1)	0.3	(0)	0.3	(0)	0.3	(0)	0.3	(0)	0.3	(0)
Site B, 2009	0.2	(0)	0.3	(0)	0.3	(0)	0.4	(0.1)	0.3	(0)	0.3	(0)	0.3	(0)

Table 5a

Results for the analysis of bulk samples from the Lena district, central Asturias, Spain: Na = material not available for analysis; nd = not determined; Not Recent = no recent manure applied; Biennial = manure applied every second year; Direct = manure applied during sampled growing season (an average of 3 to 4 replicate fields were sampled per manure level when available, with 1 SD in parentheses)

Year	Not recent		Biennial		Direct		Not recent		Biennial		Direct	
2007	Grain or pulse $\delta^{15}N$						Rachis $\delta^{15}N$					
Spelt	5.0	(2.5)	3.9	(1.1)	7.5	(2.3)	2.3	(2.0)	2.4	(1.0)	5.2	(2.6)
Broad bean	1.0		0.6	(0.6	na							
	Grain or pulse %N						Rachis %N					
Spelt	na		2.2	(0.2)	na		na		0.2	(0.0)	na	
Broad bean	4.1		3.5	(0.3)								

Table 5b

Results for the analysis of bulk samples from the Sighisoara region, Transylvania, Romania: na= material not available for analysis; nd= not determined; Not recent = no recent manure applied; Triennial = manure applied every third year; Direct = manure applied during sampled growing season (an average of 3 to 4 replicate fields were sampled per manure level when available, with 1 SD in parentheses).

Year	Not recent		Triennial		Direct		Not recent		Triennial		Direct	
2007	Grain or pulse $\delta^{15}N$						Rachis $\delta^{15}N$					
Bread wheat	2.3	(1.2)	na		4.7	(0.9)	0.1	(0.6)	na		1.9	(1.1)
Einkorn	1.4		2.7	(2.3)	3.5	(2)	-1.2		1.0	(2.6)	1.0	(1.7)
Lentil	0.0		0.9		0.5	(0.4)						
	Grain or pulse %N						Rachis %N					
Bread wheat	1.4	(0.2)	na		1.7	(0.2)	0.3	(0.0)	na		0.3	(0.0)
Einkorn	1.7		1.6		2.0	(0.2)	0.20		0.2	(0.1)	0.2	(0.0)
Lentil	4.9		3.8		4	(0.4)						

Table 5c

Results for the analysis of bulk samples from the Aliveri district of central Evvia, Greece: Low = low manure, High= high manure (an average of 3 to 4 replicate fields were sampled per manure level when available, with 1 SD in parentheses).

Year	Low		High	
2007	$\delta^{15}N$			
Broad bean	0.6	(0.4)	2.2	(1.4)
	%N			
Broad bean	4.1	(0.5)	4.2	(0.4)

Table 6

Results of two-way ANOVA statistical tests (t-test applied to Rothamsted) on cereal grain and pulse, A) $\delta^{15}N$ values and, B) %N values (F(df effect, df error) = F value, p-value. † indicates data from all years were combined because sample sizes were too small for statistical analysis on separate years, na = not applicable, * significant response at an alpha level of 0.05).

A) Grain and pulse $\delta^{15}N$ values

Site	Effect of manure	p value	Effect of crop species	p value
Rothamsted Broadbalk†	t(7.49)	<0.001*	na	
Rothamsted Hoosfield†	t(9.18)	<0.001*	na	
Askov 2007	F(1,8) = 195.34	<0.001*	F(1,8) = 4.48	0.067
Askov 2008	F(1,12) = 319.54	<0.001*	F(2,12) = 0.053	0.949
Sut. Bonington Site A 2007	F(1,18) = 24.37	<0.001*	F(2,18) = 0.54	0.594
Sut. Bonington Site B 2008	F(2,27) = 1.21	0.313	F(2,27) = 0.880	0.426
Bad Lauchstädt cereals†	F(2,15) = 57.41	<0.001*	F(1,15) = 2.71	0.120
Bad Lauchstädt pulses†	F(2,6) = 4.23	0.072	F(1,6) = 8.55	0.026*
Site and year	Effect of manure	p value	Effect of irrigation	p value
Aleppo Site A cereals 2008	F(2,15) = 18.01	<0.001*	F(2,15) = 1.30	0.301
Aleppo Site B cereals 2009	F(2,15) = 64.45	<0.001*	F(2,15) = 7.95	0.004*
Aleppo Site A pulses 2008	F(2,15) = 18.01	<0.001*	F(2,15) = 1.30	0.301
Aleppo Site B pulses 2009	F(2,15) = 7.41	0.006*	F(2,15) = 2.02	0.167

B) Grain and pulse %N values

Site	Effect of manure	p value	Effect of crop species	p value
Rothamsted Broadbalk†	t(2.08)	0.067	na	
Rothamsted Hoosfield†	t(1.93)	0.079	na	
Askov 2007	F(1,8) = 15.33	0.004*	F(1,8) = 5.97	0.040*
Askov 2008	F(1,12) = 1.73	0.213	F(2,12) = 0.457	0.644
Sut. Bonington Site A 2007	F(1,17) = 3.12	0.095	F(2,17) = 14.56	<0.001
Sut. Bonington Site B 2008	F(2,27) = 0.99	0.386	F(2,27) = 1.09	0.351
Bad Lauchstädt cereals†	F(2,15) = 0.86	0.442	F(1,15) = 0.19	0.670
Bad Lauchstädt pulses†	F(2,6) = 0.63	0.566	F(2.6) = 14.785	0.009*

Site and year	Effect of manure	p value	Effect of irrigation	p value
Aleppo Site A cereals 2008	F(2,15) = 0.97	0.401	F(2,15) = 3.49	0.057
Aleppo Site B cereals 2009	F(2,15) = 3.05	0.078	F(2,15) = 3.83	0.05*
Aleppo Site A pulses 2008	F(2,15) = 0.97	0.401	F(2,15) = 3.50	0.057
Aleppo Site B pulses 2009	F(1,15) = 0.24	0.792	F(2,15) = 1.50	0.261

Table 7

Results of two-way ANOVA tests (t-test applied to Rothamsted) on cereal rachis, A) $\delta^{15}N$ values and, B) %N values (F(df effect, df error) = F value, p-value. † indicates data from all years were combined because sample sizes were too small for statistical analysis on separate years, na = not applicable, * significant response at an alpha level of 0.05).

A) Cereal rachis $\delta^{15}N$

Site and year	Effect of manure	p value	Effect of crop species	p value
Rothamsted Broadbalk	††		na	
Rothamsted Hoosfield†	t(6.13)	0.001*	na	
Askov 2007	F(1,6) = 197.37	<0.001*	F(1,6) = 1.92	0.209
Askov 2008	F(1,12) = 321.97	<0.001*	F(2,12) = 0.68	0.527
Sut. Bonington Site A 2007	F(1,18) = 10.09	0.005*	F(2,18) = 1.63	0.223
Sut. Bonington Site B 2008	F(2,26) = 1.31	0.287	F(2,26) = 1.02	0.375
Bad Lauchstädt†	F(2,9) = 34.3	<0.001*	F(2,9) = 1.9	0.192
Site and year	Effect of manure	p value	Effect of irrigation	p value
Aleppo Site A 2008	F(2,15) = 3.28	0.066	F(2,15) = 0.97	0.403
Aleppo Site B 2009	F(2,15) = 31.47	<0.001*	F(2,15) = 0.91	0.422

B) Cereal rachis %N

Site and year	Effect of manure	p value	Effect of crop species	p value
Rothamsted Broadbalk	††		na	
Rothamsted Hoosfield†	t(0.95)	0.380	na	
Askov 2007	F(1,7) = 0.801	0.405	F(1,7) = 2.96	0.136
Askov 2008	F(1,12) = 0.19	0.671	F(1,12) = 17.96	<0.001*
Sut. Bonington Site A 2007	F(1,17) = 4.17	0.057	F(2,17) = 19.44	<0.001*
Sut. Bonington Site B 2008	F(2,27) = 0.21	0.813	F(2,27) = 2.78	0.080
Bad Lauchstädt†	F(2,9) = 0.19	0.834	F(2,9) = 0.09	0.773
Site and year	Effect of manure	p value	Effect of irrigation	p value
Aleppo Site A 2008	F(1,12) = 0.05	0.956	F(1,12) = 0.39	0.678
Aleppo Site B 2009	F(2,15) = 0.08	0.922	F(2,15) = 0.70	0.512

Figure 1

Map showing location of sites: 1) Sutton Bonington, 2) Rothamsted, 3) Askov, 4) Bad Lauchstädt, 5) Asturias, 6) Sighisoara, 7) Evvia and 8) Aleppo.

Figure 2

$\delta^{15}N$ values for bulk samples of grain from the long-term experimental plots at: a) Rothamsted (no replicates per plot), b) Askov (average of 3 replicates per plot with 1 SD), c) Bad Lauchstädt (no replicates per plot).

Figure 3

$\delta^{15}N$ values for bulk samples of grain from the short-term experimental plots at: a) Sutton Bonington: SB-Site A, 2007, b) Sutton Bonington: SB-Site B, 2008, b) Aleppo: AL-Site A, 2008, c) Aleppo: AL-Site B, 2009. Average of 3 replicate plots per treatment, with 1 SD.

Figure 4

$\delta^{15}N$ values for bulk samples of pulses from, a) long-term experimental plots at Bad Lauchstädt (no replicates per plot), and b) short-term experimental plots at Aleppo (average of three replicate plots per treatment, with 1 SD).

Figure 5

The $\delta^{15}N$ values for bulk samples of grain and pulses from the traditional farming studies at: a) Asturias, b) Sighisoara, c) Evvia. No replicates per field at each site.

Figure 6

The relationship between $\delta^{15}N$ values of cereal grain and rachis. The r value reports the Pearson product-moment correlation coefficient and P value, at a significance level of 0.05.

Figure 7

The relationships between a) unmanured and b) FYM 2 and SLU 1½ (Askov) manured cereal $\delta^{15}N$ values and mean annual precipitation. The r values report the Pearson product-moment correlation coefficients and P values, at a significance level of 0.05.

Figure 1- Map showing location of sites

1. Sutton Bonington
2. Rothamsted
3. Askov
4. Bad Lauchstädt
5. Asturias
6. Sighisoara
7. Evvia
8. Aleppo

Figure 2

$\delta^{15}N$ values for bulk samples of grain from the long-term experimental plots at: a) Rothamsted (no replicates per plot), b) Askov (average of 3 replicates per plot with 1 SD), c) Bad Lauchstädt (no replicates per plot).

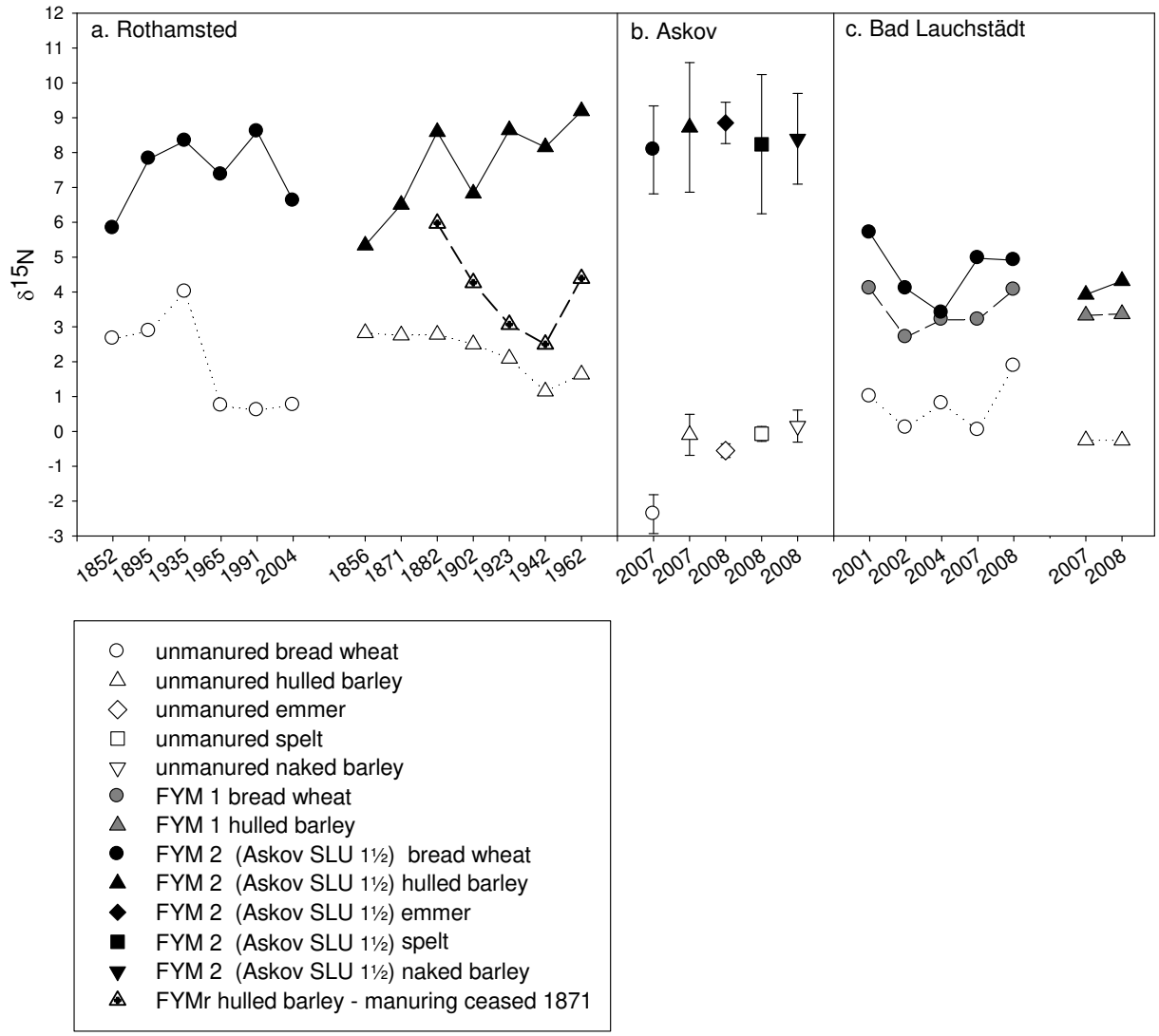


Figure 3

$\delta^{15}N$ values for bulk samples of grain from the short-term experimental plots at: a) Sutton Bonington: SB-Site A, 2007, b) Sutton Bonington: SB-Site B, 2008, b) Aleppo: AL-Site A, 2008, c) Aleppo: AL-Site B, 2009. Average of 3 replicate plots per treatment, with 1 SD.

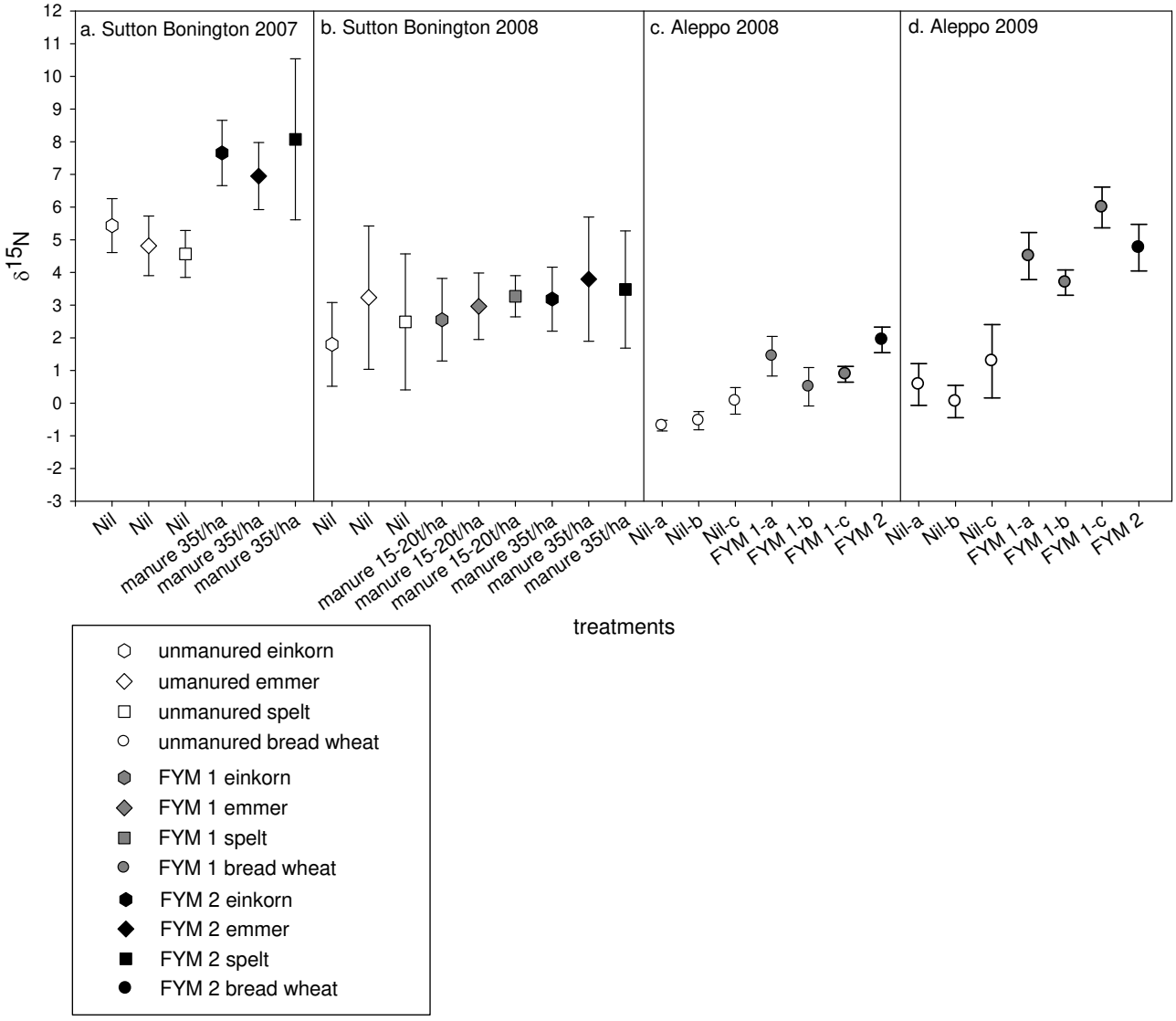


Figure 4

$\delta^{15}N$ values for bulk samples of pulses from, a) long-term experimental plots at Bad Lauchstädt (no replicates per plot), and b) short-term experimental plots at Aleppo (average of three replicate plots per treatment, with 1 SD).

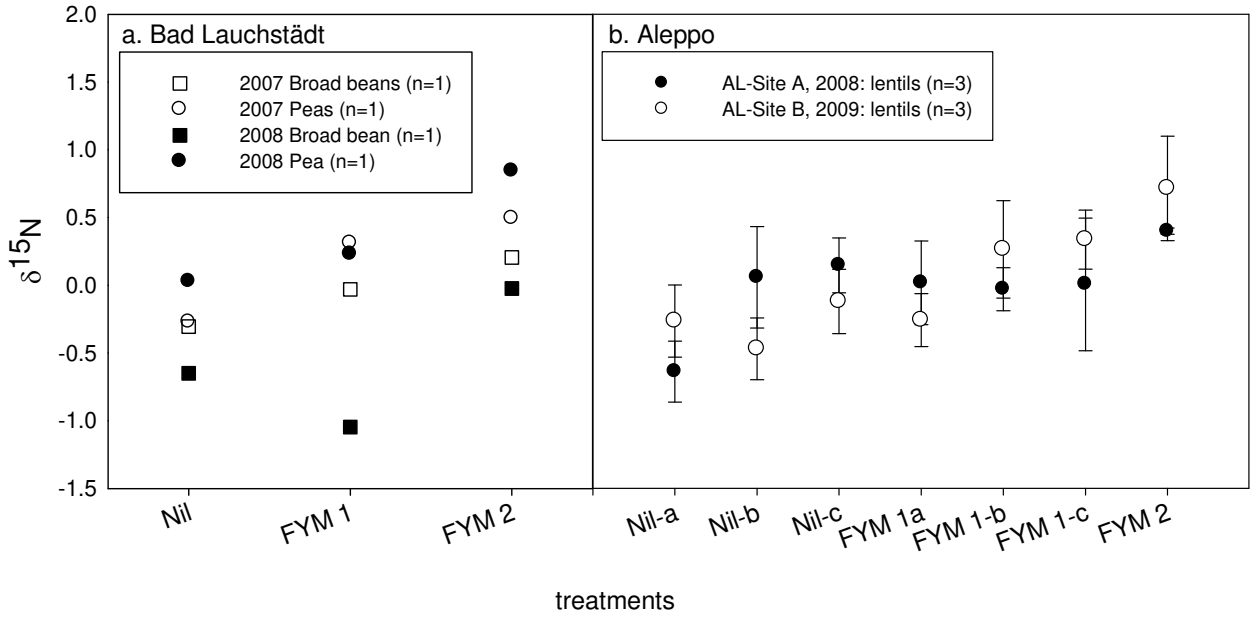


Figure 5

The $\delta^{15}N$ values for bulk samples of grain and pulses from the traditional farming studies at: a) Asturias, b) Sighisoara, c) Evvia. No replicates per field at each site.

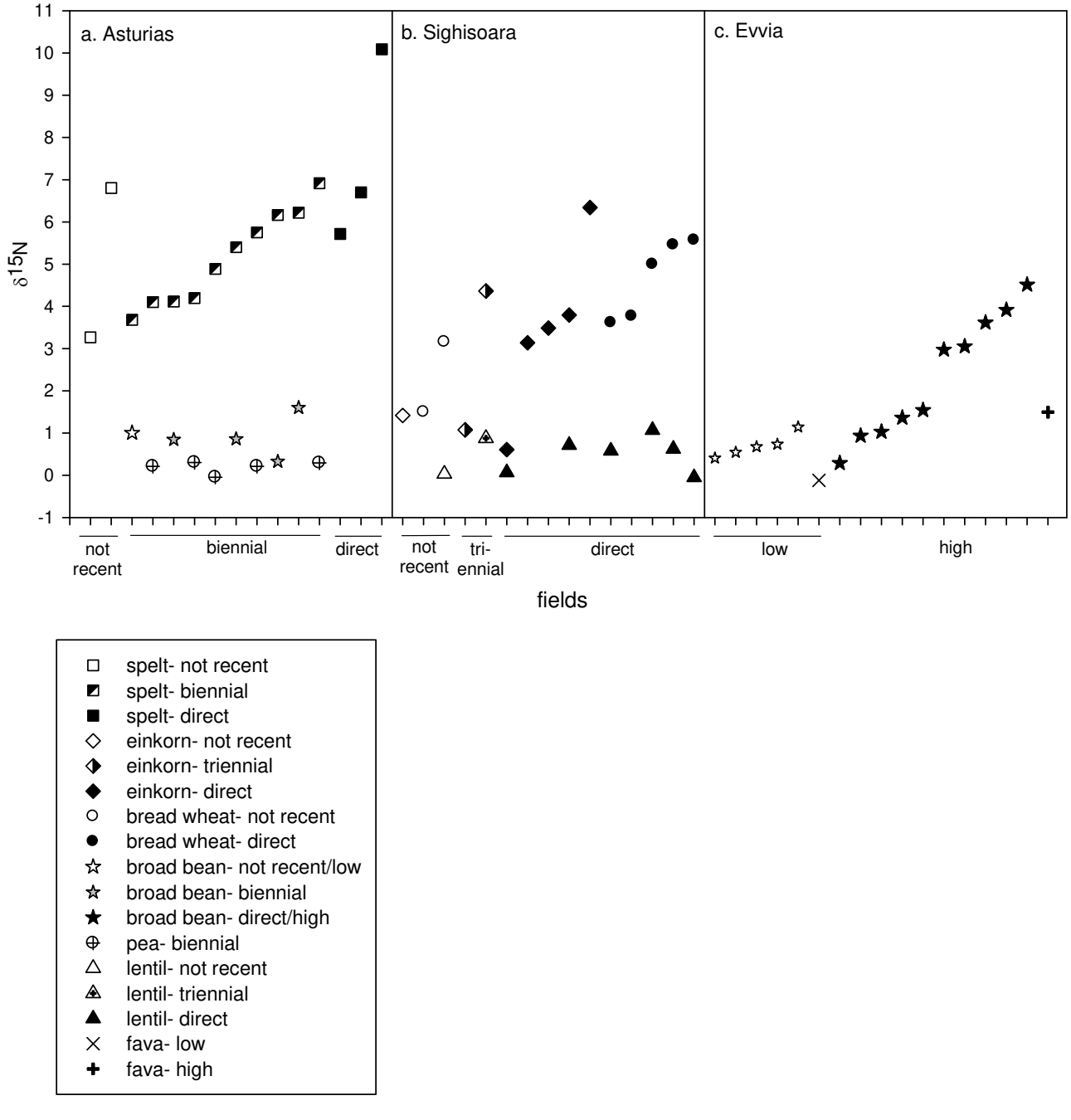


Figure 6

The relationship between $\delta^{15}N$ values of cereal grain and rachis. The r value reports the Pearson product-moment correlation coefficient and P value, at a significance level of 0.05.

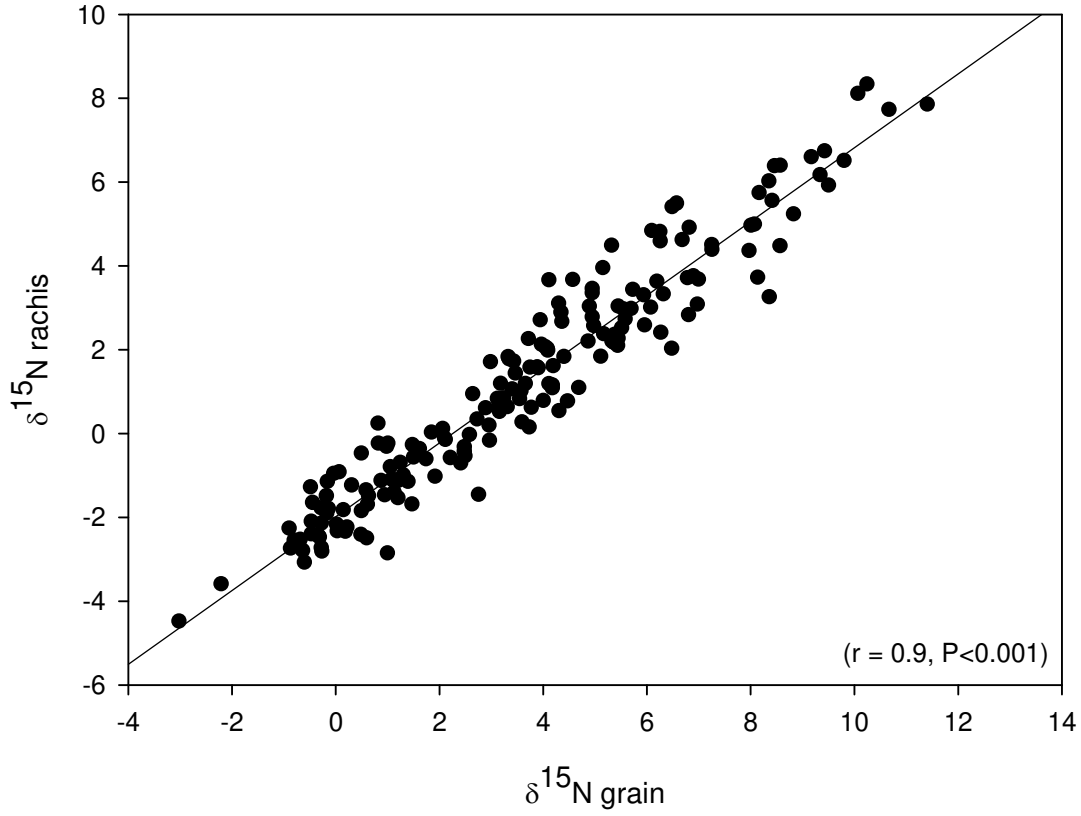
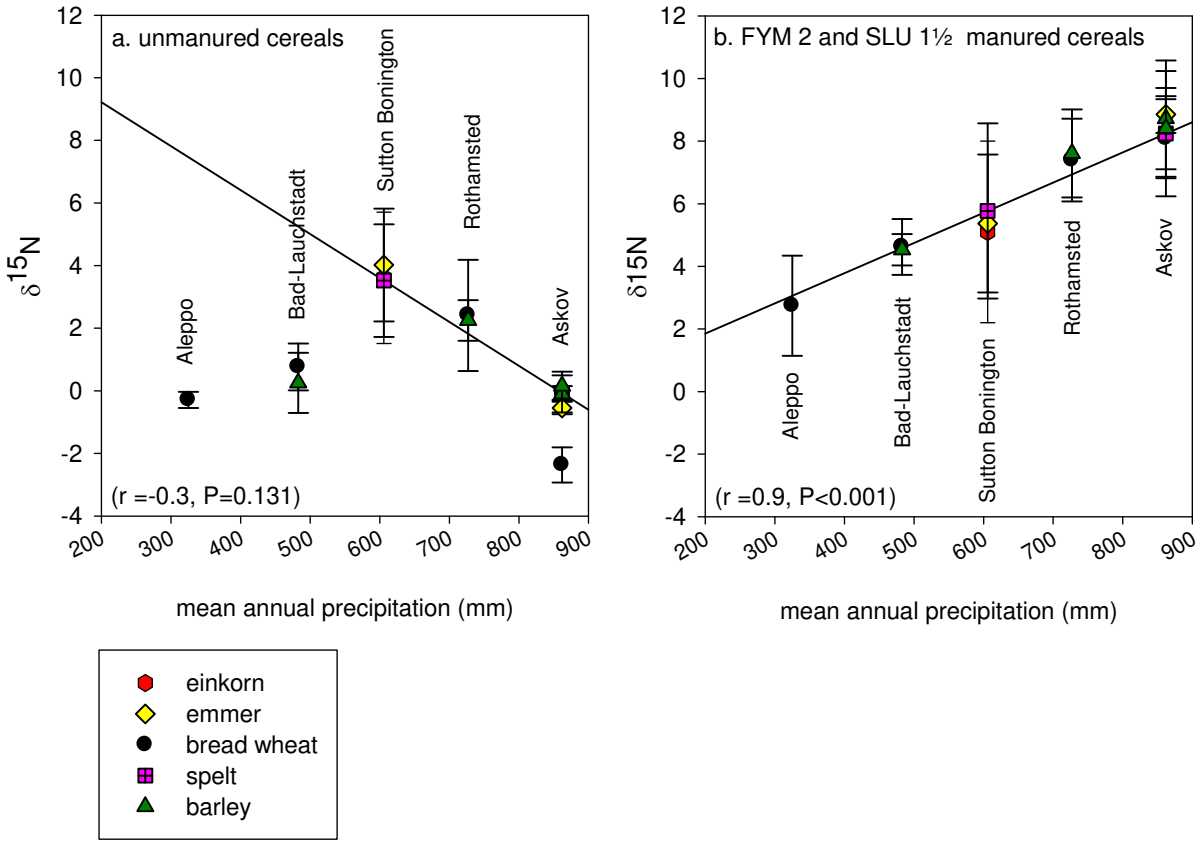


Figure 7

The relationships between a) unmanured and b) FYM 2 and SLU 1½ (Askov) manured cereal $\delta^{15}N$ values and mean annual precipitation. The r values report the Pearson product-moment correlation coefficients and P values, at a significance level of 0.05.