Rothamsted Repository Download

A - Papers appearing in refereed journals

Senbayram, M., Dixon, E. R., Goulding, K. W. T. and Bol, R. 2008. Long-term influence of manure and mineral nitrogen applications on plant and soil 15N and 13C values from the Broadbalk Wheat Experiment. *Rapid Communications In Mass Spectrometry.* 22 (11), pp. 1735-1740.

RAPID COMMUNICATIONS IN MASS SPECTROMETRY
Rapid Commun. Mass Spectrom. 2008; **22**: 1735–1740
Published online in Wiley InterScience (www.interscience.wiley.com) DOI: 10.1002/rcm.3548

RCM

Long-term influence of manure and mineral nitrogen applications on plant and soil ^{15}N and ^{13}C values from the Broadbalk Wheat Experiment†

Mehmet Senbayram[1,3]*, Liz Dixon[1], Keith W. T. Goulding[2] and Roland Bol[1]

[1]Cross Institute Programme for Sustainable Soil Function (SoilCIP), North Wyke Research, Okehampton EX20 2SB, UK
[2]Cross Institute Programme for Sustainable Soil Function (SoilCIP), Rothamsted Research, Harpenden AL5 2JK, UK
[3]Plant Nutrition and Soil Science Department, Kiel CAU, Olshausenstr. 40, 24118 Kiel, Germany

Received 20 March 2008; Accepted 21 March 2008

The Broadbalk Wheat Experiment at Rothamsted Research in the UK provides a unique opportunity to investigate the long-term impacts of environmental change and agronomic practices on plants and soils. We examined the influence of manure and mineral fertiliser applications on temporal trends in the stable N (^{15}N) and C (^{13}C) isotopes of wheat collected during 1968–1979 and 1996–2005, and of soil collected in 1966 and 2000. The soil $\delta^{15}N$ values in 1966 and 2000 were higher in manure than the mineral N supplied soil; the latter had similar or higher $\delta^{15}N$ values than non-fertilised soil. The straw $\delta^{15}N$ values significantly decreased in all N treatments during 1968 to 1979, but not for 1996–2005. The straw $\delta^{15}N$ values decreased under the highest mineral N supply (192 kg N ha^{-1} year^{-1}) by 3‰ from 1968 to 1979. Mineral N supply significantly increased to straw $\delta^{13}C$ values in dry years, but not in wet years. Significant correlations existed between wheat straw $\delta^{13}C$ values with cumulative rainfall (March to June). The cultivar *Hereward* (grown 1996–2005) was less affected by changes in environmental conditions (i.e. water stress and fertiliser regime) than *Cappelle Desprez* (1968–1979). We conclude that, in addition to fertiliser type and application rates, water stress and, importantly, plant variety influenced plant $\delta^{13}C$ and $\delta^{15}N$ values. Hence, water stress and differential variety response should be considered in plant studies using plant $\delta^{13}C$ and $\delta^{15}N$ trends to delineate past or recent environmental or agronomic changes. Copyright © 2008 John Wiley & Sons, Ltd.

Nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) stable isotopes are widely used to study the responses of plants and soils to the long-term impacts of environmental change and agricultural practices.[1–3] The availability of data and samples from long-continued field experiments represents an invaluable resource for the testing and verification of such research. The Broadbalk Wheat Experiment (established in 1843) provides a unique opportunity to examine the long-term effects of animal manure and mineral nitrogen (N) fertiliser on plant and soil $\delta^{15}N$ and $\delta^{13}C$ contents.[4–6]

Plant and soil nitrogen isotope ($\delta^{15}N$) contents may be influenced by soil and plant biochemical processes, morphological plant traits and abiotic factors. Shearer and Kohl reported that plant $\delta^{15}N$ values generally reflect the source of the plant available nitrogen.[7] Various nitrogen sources applied in agricultural systems differ in their $\delta^{15}N$ values and therefore modify both soil and plant $\delta^{15}N$ values.[8,9] However, the differential $\delta^{15}N$ discrimination of several plant and soil processes complicates the interpretation of results with respect to sources.[8–10] Generally the long-term application of manure, which is relatively enriched in ^{15}N, leads to higher plant and soil $\delta^{15}N$ values than in unamended (no manure) control plots.[2] However, changes in plant and soil $\delta^{15}N$ values are less consistent when relatively ^{15}N-depleted mineral (inorganic) N fertilisers are applied. Indeed, studies have shown that long-term applications of mineral fertiliser can decrease, increase or have no significant effect on plant and soil $\delta^{15}N$ values compared with unamended (no added fertiliser N) control treatments.[2,9]

Plant carbon ($\delta^{13}C$) isotope signatures may be used as an indicator of long-term transpiration efficiency and the ratio of net photosynthesis to stomatal conductance (intrinsic water use efficiency) during assimilation.[11,12] Effects of nitrogen supply and drought on intrinsic water use efficiency and thus on plant $\delta^{13}C$ values have been reported.[4,12] Hubick[13] found no nitrogen effect on (peanut) plant $\delta^{13}C$, but increases of plant $\delta^{13}C$ under high compared with low rates of nitrogen fertiliser were observed.[14–16] The effects of nitrogen on plant $\delta^{13}C$ are strongly influenced by other environmental variables.[4,17] Jenkinson and co-authors reported positive effects of nitrogen supply on plant $\delta^{13}C$ in dry but not in wet years.[4] However, most of the above-mentioned studies compared only low and high

**Correspondence to*: M. Senbayram, Plant Nutrition and Soil Science Department, Kiel CAU, Olshausenstr. 40, 24118 Kiel, Germany.
E-mail: mehmetsenbayram6@yahoo.co.uk
†Presented at the annual meeting of the Stable Isotopes Mass Spectrometry Users' Group (SIMSUG), 20–22 June, 2007, hosted by the Institute for Research on the Environment and Sustainability (IRES) and the School of Civil Engineering and Geosciences, Newcastle University, UK.
Contract/grant sponsor: IGER North Wyke.

nitrogen supply. The question remains whether or not there is a consistent correlation between mineral nitrogen supply and plant $\delta^{13}C$ over a range of nitrogen application which might explain more about the relation between leaf physiology and environmental changes, especially comparing various plant species and cultivars (here wheat cultivars).

Ecophysiological data have been used to study the responses of plants to past environmental changes.[5] However, as far as we are aware, there are very few long-term data sets available which report on the responses of plant species (here wheat) to environmental changes or anthropogenic activity (such as fertiliser or manure applications). Long-term climate, environmental or agronomic reconstructions using plant $\delta^{13}C$ or $\delta^{15}N$ signatures are, however, an invaluable and informative way of examining such responses.

We therefore examined the $\delta^{15}N$ and $\delta^{13}C$ values of wheat straw and grain, together with some additional soil samples, for two periods (1968–1979 and 1996–2005) with respect to the effects of: (i) a range of mineral N fertiliser application rates (0 to 192 kg N ha^{-1} year^{-1}) and manure (35 t ha^{-1} year^{-1}; containing, on average, 257 kg N ha^{-1} year^{-1}, predominantly as organic N), and (ii) two different wheat cultivars, cv. *Cappelle Desprez* (grown from 1968 to 1979) and cv. *Hereward* (grown from 1996 to 2005).

EXPERIMENTAL

The Broadbalk Wheat Experiment at Rothamsted Research in the UK occupies approximately 5 ha of land which is now divided into 10 sections to test different crop sequences, including continuous wheat. Different combinations of fertiliser and manure are applied every year; some treatments date back to the start of the experiment in 1843. The soil is a silty clay loam, producing good wheat yields of up to 11 t ha^{-1}. Different varieties of winter wheat, selected from the main varieties used in the UK, have been grown over the years. Wheat is generally sown in October and harvested in August. Archived soil, wheat grain and straw samples were taken from the sections of the experiment on which wheat is grown year after year. Subsamples of grain and straw from harvests were dried at 80°C for 16 h before being archived in sealed glass jars or tin boxes. For detailed information about the experiment, see Poulton,[18] the web pages of the Electronic Rothamsted Archive[19] and Johnston and Garner.[20] We analysed samples from six treatments; five were from plots receiving PKMg fertiliser and variable rates of mineral N fertiliser (0, 48, 96, 144, 192 kg N ha^{-1}) and the sixth from plots receiving manure (35 t ha^{-1} which was on average 257 kg N ha^{-1}). Two time periods were considered; 1968–1979 when cv. *Cappelle Desprez* was grown and 1996–2005 when cv. *Hereward* was grown. Weather data was taken from the Rothamsted weather station.

The plant and soil $\delta^{13}C$ and $\delta^{15}N$ values were analysed at the North Wyke Research, Okehampton, UK, using a NA 1500 elemental analyser (Carlo Erba, Milan. Italy) and an automated continuous flow ANCA 20/20SL system (Europa, Crewe, UK). The natural abundance values were expressed as δ values, which represent the ratios of $^{13}C/^{12}C$ or $^{15}N/^{14}N$ relative to the international VPDB and AIR standard, respectively, defined as:

$$\delta^{13}C = [(\text{atom}\%^{13}C_{\text{sample}} - \text{atom}\%^{13}C_{\text{VPDB}})/\text{atom}\%^{13}C_{\text{VPDB}}] \times 1000$$

$$\delta^{15}N = [(\text{atom}\%^{15}N_{\text{sample}} - \text{atom}\%^{15}N_{\text{AIR}})/\text{atom}\%^{15}N_{\text{AIR}}] \times 1000$$

The analytical precisions of the $\delta^{13}C$ and $\delta^{15}N$ measurements were <0.1 and <0.2‰, respectively.

The results were analysed with analysis of variance (ANOVA) using the SPSS v.13.0 software (SPSS, Chicago, IL. USA). Pairwise comparisons between the treatments were performed by comparing treatment means with the least significant difference (LSD) procedure. The correlations between the parameters were studied by calculating Pearson's correlation coefficient (r). Multiple comparisons between the treatment means were performed with Tukey's HSD tests.

RESULTS AND DISCUSSION

Influence of mineral and manure N inputs on soil $\delta^{15}N$ and $\delta^{13}C$

The soil $\delta^{15}N$ values in 1966 and 2000 are given in Table 1. Manure-amended soil had a $\delta^{15}N$ of at least 1.9‰ (in 1966) and 1.7‰ (in 2000) higher than soils that received mineral fertiliser or no fertiliser (control). This would be expected because of the higher $\delta^{15}N$ values of manure (5.4‰ ± 0.7, pooled over 6-year samples). Organic fertilisers tend to be enriched in $\delta^{15}N$ compared with the plant materials ingested by animals due to isotopic discrimination within the animal and microbial processes and NH_3 volatilisation after excretion.[21] Similar results have been reported by others.[22–25]

The mineral N-amended soil had similar or higher $\delta^{15}N$ values than the control in both years (except N_3PKMg) (Table 1). Although soil samples were taken from different sections of the Broadbalk experiment, it remains unexpected to have (in both periods) higher $\delta^{15}N$ values in soil amended with mineral N than in the non-fertilised soil. This is surprising as mineral N fertiliser has $\delta^{15}N$ values close to the atmospheric value ($\delta^{15}N = 0$‰) and these are generally lower than the $\delta^{15}N$ values of the soil to which it is supplied. The $\delta^{15}N$ enrichment from 1966 to 2000 in the fertilised soils might have been due to fractionation against ^{15}N compared

Table 1. Effects of organic manure and mineral fertiliser application on soil $\delta^{13}C$ and $\delta^{15}N$ values in 1966 and 2000

	1966		2000	
Treatment	$\delta^{13}C$‰	$\delta^{15}N$‰	$\delta^{13}C$‰	$\delta^{15}N$‰
N_0PKMg	−21.5	2.1	−24.6	3.6
N_1PKMg	−24.2	2.1	−25.0	4.6
N_2PKMg	−24.7	2.7	−25.4	4.3
N_3PKMg	−25.0	1.9	−25.6	3.6
N_4PKMg*	−22.2	3.3	−24.7	4.0
Manure (257 kg N ha^{-1})	−24.3	5.2	−27.3	6.3

Note: N_0–N_4 = 0, 48, 96, 144, 192 kg N ha^{-1}.
*N_4 since 1968 only, previously 48 kg N ha^{-1} only.

DOI: 10.1002/rcm

with ^{14}N during mineralisation, nitrification, volatilisation, and plant uptake. These processes lead to N losses which are relatively enriched in ^{14}N, especially from the active part of the organic N pool in the soil. Thus the soil pool becomes relatively enriched in ^{15}N over time.[7] Previous research on the Broadbalk experiment showed that losses of inorganic N increase with rates of mineral N or manure application.[26] Robinson estimates the N isotope fractionations for major N cycle processes as 40–60‰ for NH_3 volatilisation, 35–60‰ for nitrification, 28–33‰ for denitrification, and 0–19‰ for NO_3^- and 9–18‰ for NH_4^+ assimilation by plants.[10] Therefore, increases in $\delta^{15}N$ values of soil are to be expected.[10] Högberg reported that use of large amounts of mineral fertiliser increased the soil $\delta^{15}N$ values in a long-term forest experiment,[17] because of higher N losses (relatively enriched in ^{14}N compared with ^{15}N) from the forest system and the discrimination of light N isotopes during soil processes which lead to N losses from the system.[17,27] However, others have observed that long-term application of mineral fertiliser reduces the soil $\delta^{15}N$ content.[28–30]

The soil $\delta^{13}C$ values in 1966 were similar regardless of treatment (Table 1). However, soil $\delta^{13}C$ values were clearly lower for manure-amended soils than for mineral N-amended and non-fertilised soils in 2000. This is expected because of the lower $\delta^{13}C$ values of manure (–27.0% $\pm$ 0.6, pooled over 6-year samples). Interestingly, Gerzabek and co-authors did report that the addition of animal manure over a period of 37 years caused a small but significant decrease in soil $\delta^{13}C$ contents,[31] but contrary results have also been reported in long-term field experiments.[28]

Influence of mineral and manure N inputs on plant $\delta^{15}N$ and $\delta^{13}C$

Wheat grown on manure-amended soil always had higher $\delta^{15}N$ values in straw and grain than that grown on non-fertilised and mineral N-amended soil (Fig. 1). The consistently higher $\delta^{15}N$ values of manure-amended straw samples may primarily be attributed to the application of ^{15}N-enriched N manure. Similar, positive effects of organic material amendment on plant $\delta^{15}N$ values have been reported.[23,32]

Variation in the $\delta^{15}N$ values of plants is generally hard to explain, because they are affected not only by fertiliser N, but also by for example: (i) other N sources in soil, (ii) the depth of soil from which N is taken up, and (iii) the atmospheric deposition of ammonium. As can be seen in Fig. 1, straw from mineral N-amended soils had higher values of $\delta^{15}N$ than that from non-fertilised plots, especially in the 1968 to 1979 period. Again, as stated in the previous section, this is surprising as mineral N fertiliser generally has $\delta^{15}N$ values (~0‰ $\delta^{15}N$) lower than the soil to which it is supplied. This may suggest that plant $\delta^{15}N$ values do not always directly reflect the $\delta^{15}N$ signature of fertiliser N applied to the soil. Choi and co-authors reported that the $\delta^{15}N$ signature of maize grown in a glasshouse reflected the fertiliser $\delta^{15}N$ value in the early stage of plant growth, but that in later stages the $\delta^{15}N$ values increased significantly.[32] They hypothesised that this was because of the increase in the $\delta^{15}N$ value of exchangeable N pool in the soil, linked to the N losses, which discriminated against the heavier

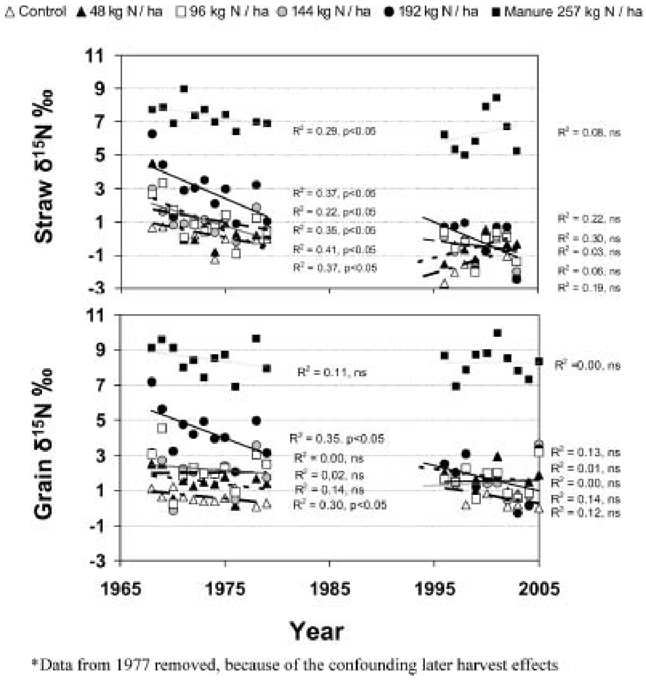

Figure 1. Effects of mineral N fertiliser and manure supply on straw and grain $\delta^{15}N$ values during the 1968–1979 and 1996–2005 periods.

^{15}N compared with ^{14}N.[27,32] Results from the long-term experiment of Meints and co-authors also agreed with our findings and showed the positive effects of higher mineral N supply on leaf $\delta^{15}N$ values of maize.[9] Another explanation for the results in the present study could be that at higher rates of fertiliser N application, increasing amounts of fertiliser N exchange with soil organic N, which has a higher $\delta^{15}N$.[9] Interestingly, the pronounced effect of higher mineral N applications on the $\delta^{15}N$ values of straw and grain was much clearer in the period 1968–1979 than in 1996–2005 (Fig. 2). This may be related to the different fertiliser source used in each period; N was applied as ammonium nitrate after 1986, but as calcium ammonium nitrate in 1968–1979.

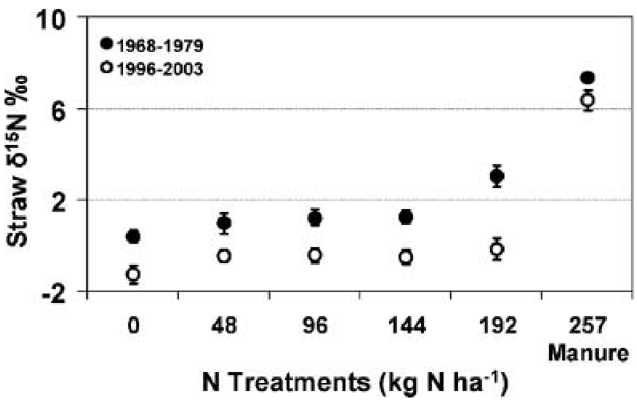

Figure 2. Relationship between mean of straw $\delta^{15}N$ values and N treatments. Data pooled for each time period in each treatment. Error bars show the standard error of each treatment.

DOI: 10.1002/rcm

RCM

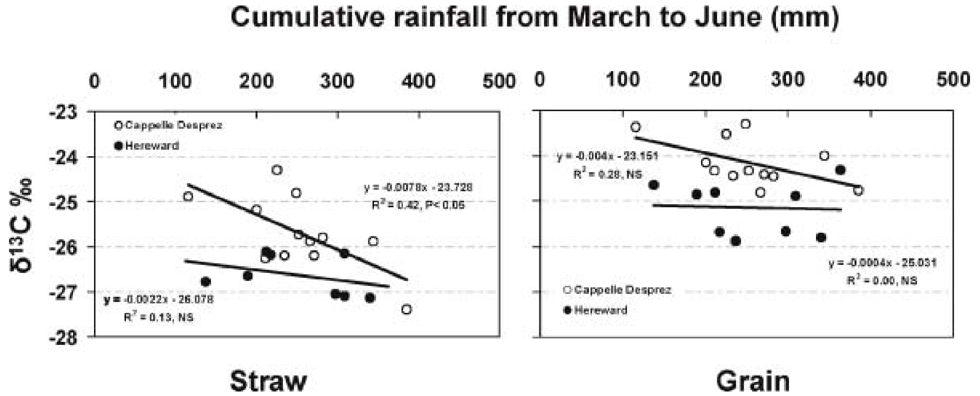

Figure 3. Relationship between mean of straw and grain $\delta^{13}C$ values (pooled over all treatments) and cumulative rainfall (March to end of June) in the periods from 1968–1979 (cv. *Cappelle Desprez*) and 1996–2003 (cv. *Hereward*).

Unfortunately, there are no fertiliser samples available for analysis to check this hypothesis.

Grain samples also showed the same response as straw to organic and mineral N fertiliser amendment; however, grain $\delta^{15}N$ values were always higher than straw $\delta^{15}N$ values (Fig. 1). This may be explained by discrimination within the plant during N uptake, translocation into leaves and stem and later remobilisation into the grain. Another explanation could be that grain $\delta^{15}N$ values reflects the $\delta^{15}N$ values of soil exchangeable N values during the grain filling period which might increase over time (see previous paragraph).

The straw and grain samples decreased in their $\delta^{15}N$ contents from 1968 to 1979 in all treatments, but the decreases were mainly significant for the straw samples (Fig. 1). Straw $\delta^{15}N$ depletion was larger in the medium and high mineral N treatments than in the manure-amended and the non-fertilised treatments. The straw $\delta^{15}N$ values decreased by more then 3‰ from 1968 to 1979 on plots receiving the largest amount of mineral nitrogen (192 kg N ha^{-1} year^{-1}), but by less than 2‰ in the manure-amended and the control treatments with regards to the fitted line. There was no significant trend found in straw and grain $\delta^{15}N$ values during the 1996–2005 period (Fig. 1). Comparing soil and straw $\delta^{15}N$ values, the straw $\delta^{15}N$ values were much closer to the soil $\delta^{15}N$ value in the period 1968–1979 than in 1996–2005. Both the straw and the grain $\delta^{15}N$ values were less than the soil $\delta^{15}N$ values in the 1996–2005 period. This partly agrees with the report by Meints and co-authors that corn and soybean leaf $\delta^{15}N$ values were considerably lower than soil $\delta^{15}N$ values.[9]

We observed that the wheat straw was depleted in $\delta^{13}C$ compared with grain in both periods (Fig. 3). This was ascribed to a higher content of ^{13}C-depleted compounds (cellulose and lignin) in the straw and of ^{13}C-enriched proteins and starch in the grains.[30] Similar findings were also reported by Zhao and co-authors[5] and Farquhar and Richards.[33]

Carbon isotope discrimination reflects the time-integrated intercellular concentration of CO_2, and the positive effect of N supply on $\delta^{13}C$ has been widely observed (see Introduction). Higher $\delta^{13}C$ values in crops receiving mineral N than in

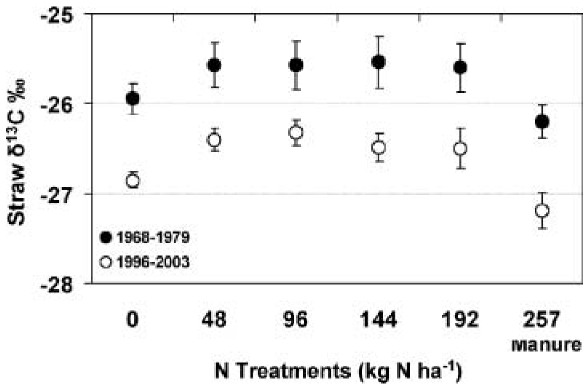

Figure 4. Relationship between mean of straw $\delta^{13}C$ values and N treatments. Data pooled for each time period in each treatment. Error bars show the standard error of each treatment.

non-fertilised crops indicate a higher photosynthetic capacity.[34,35] Jenkinson and co-authors reported that there was a large difference between $\delta^{13}C$ in N-fertilised and non-fertilised plants, but they only compared low and high N supply, whilst we compared four mineral nitrogen rates (plus a zero N and a manure treatment).[4] Our results agreed with those studies in which it was reported that N supply had a positive effect on the $\delta^{13}C$ values of straw (Fig. 4).[14–16] However, the difference was only observed between no N and N treatments; there were no differences between the different rates of N, as observed in previous study.[36] We did not see any significant effects of manure on straw $\delta^{13}C$ values (Fig. 4), in line with our earlier research which had reported that only mineral N and not manure increased $\delta^{13}C$ in barley grain.[28]

Cumulative rainfall, wheat variety and plant $\delta^{13}C$ and $\delta^{15}N$

The differences between non-fertilised and mineral N treatments varied greatly from year to year depending on water availability, with larger differences in straw $\delta^{13}C$ in dry years than in wet years. The correlation between

DOI: 10.1002/rcm

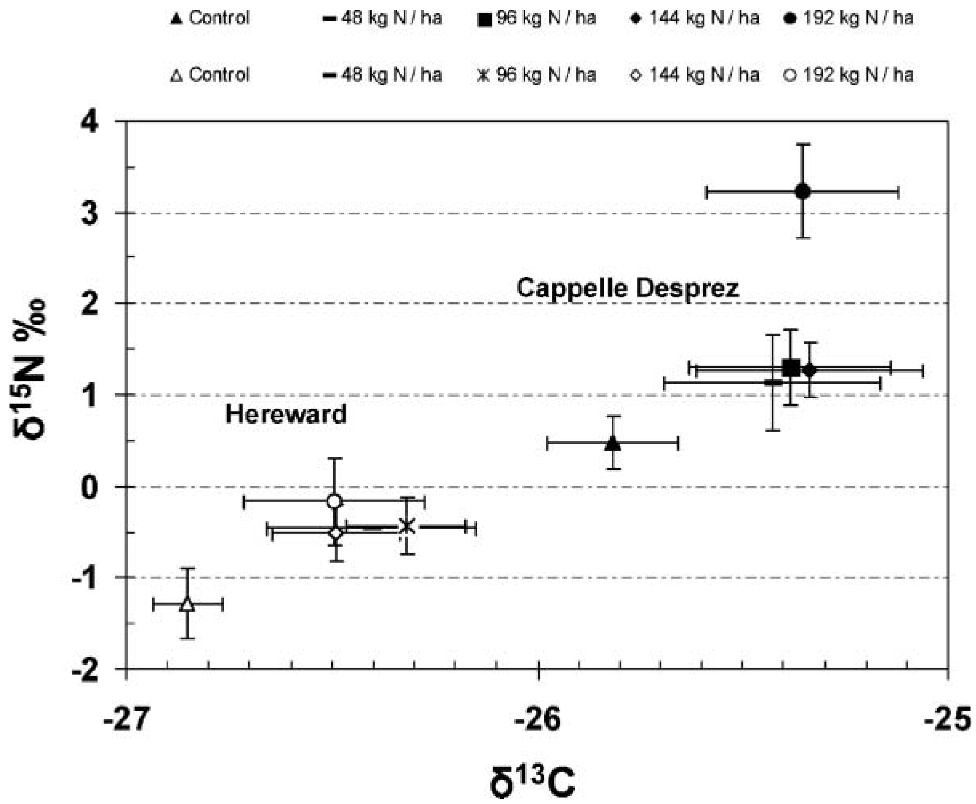

Figure 5. The $\delta^{15}N$ and $\delta^{13}C$ values of straw for two time periods under different rates of mineral N supply (kg N ha^{-1}) (black shapes: 1968–1979; open shapes: 1996–2003).

$D\delta^{13}C$ ($D\delta^{13}C$ = difference in $\delta^{13}C$ between the soils receiving no N and mineral N) and cumulative rainfall (March to June) increased with the increasing rate of mineral N fertiliser applied. The R values from the correlation of $D\delta^{13}C$ and cumulative rainfall were −0.23, −0.52, −0.49, −0.76** in 1968–1979 and −0.23, −0.30, −0.32, −0.56 in 1996–2003 for N1, N2, N3, N4 (** indicates significance at 0.001 level). Clay and co-authors reported that positive effects of N nutrition on $\delta^{13}C$ values resulted from a combination of factors.[36] First, adding N to N-deficient plants increases the machinery of photosynthesis, which in turn increases the $\delta^{13}C$ of carboxylation because of less discrimination. Secondly, adding N to N-deficient plants tends to increase biomass production, which increases water requirements, so stomatal closure occurs because of water stress.[36] Our results agree with these proposed mechanisms, especially with the second suggestion by Clay and co-authors, because the N effect on $\delta^{13}C$ that we observed was more pronounced in dry years. There was also a significant negative correlation between straw $\delta^{13}C$ values and the cumulative rainfall from March to June in the period 1968–1979 (cv. *Cappelle Desprez*), but no significant relation was found in 1996–2003 (cv. *Hereward*) (Fig. 3). The differences in response to rainfall between the two periods were also observed for the control and manure treatments. Clearly, comparing the two varieties, the newer cultivar, *Hereward*, had a lower response to rainfall than the older cultivar *Cappelle Desprez*, independent of fertiliser treatment (Fig. 3). However, straw $\delta^{15}N$ values increased when the wheat harvest was delayed, especially for a very late harvest in September, 1977, which induced much higher $\delta^{15}N$ values. The pronounced influence of harvest dates was not found for grain $\delta^{13}C$ values.

Mineral N application increased the straw $\delta^{13}C$ and $\delta^{15}N$ values (Fig. 5) in both periods, but there were clear differences in straw $\delta^{13}C$ and $\delta^{15}N$ between the two periods and varieties (Fig. 5). Rebetzke and co-authors reported that $\delta^{13}C$ is genetically correlated with yield for wheat grown in water-limited environments, and different varieties may differ in $\delta^{13}C$ signature.[37] Our findings agree, as we found clear differences in $\delta^{13}C$ values in straw when comparing the two sampling periods and different varieties for all treatments (Fig. 5). The pronounced difference in $\delta^{13}C$ values, even for non-fertilised plots, means that the newer variety *Hereward* has lower (more negative) $\delta^{13}C$ values relative to the fitted line in Fig. 3, which might be due to a higher time-integrated stomatal conductance.

Changes in plant $\delta^{13}C$ signatures may reflect the changes in atmospheric CO_2 concentrations over the last two centuries.[5] For archived grain and straw samples from unfertilised plots on Broadbalk, Zhao and co-authors found that $\delta^{13}C$ decreased by 2.5–2.8‰ from the mid-1960s to 1997. This was partly attributed to the dilution of atmospheric $^{13}CO_2$ by the release of ^{13}C-depleted CO_2 from global losses of soil organic matter, burning of plant biomass and the use of fossil energy. We concluded from our study that water stress and, also importantly, plant variety influence the plant $\delta^{13}C$ and $\delta^{15}N$ values (Fig. 5). Hence, this differential variety response should be considered in any studies using plant $\delta^{13}C$ and $\delta^{15}N$ to reconstruct or assess past or recent environmental or agronomic changes.

DOI: 10.1002/rcm

Acknowledgements

This work was partly funded by a grant awarded to M. Senbayram by IGER North Wyke. IGER and Rothamsted Research are grant-aided by the Biotechnology and Biological Science Research Council (BBSRC). We thank Dan Dhanoa for his statistical advice. We also acknowledge Paul Poulton, Klaus Dittert and two anonymous reviewers for their constructive comments.

REFERENCES

1. Bol R, Moering J, Kuzyakov Y, Amelung W. *Rapid Commun. Mass Spectrom.* 2003; **17**: 2585.
2. Lobe I, Bol R, Du Preez CC, Amelung W. *Soil Biol. Biochem.* 2005; **37**: 1898.
3. O'Brien BJ, Stout JD. *Soil Biol. Biochem.* 1978; **10**: 309.
4. Jenkinson DS, Coleman K, Harkness DD. *Plant Soil* 1995; **171**: 365.
5. Zhao FJ, Spiro B, McGrath SP. *Oecologia* 2001; **128**: 336.
6. Jenkinson DS, Harkness DD, Vance ED, Adams DE, Harrison AF. *Soil Biol. Biochem.* 1992; **24**: 295.
7. Shearer G, Kohl DH. *Aust. J. Plant Phys.* 1986; **13**: 699.
8. Högberg P, Johannisson C. *Plant Soil* 1993; **157**: 147.
9. Meints VW, Boone LV, Kurtz LT. *J. Environ. Quality* 1975; **4**: 486.
10. Robinson D. *Trends in TREE Ecology & Evolution* 2001; **16**: 153.
11. Farquhar GD, Ehleringer JR, Hubick KT. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 1989; **40**: 503.
12. Condon AG, Richards RA, Farquhar GD. *Aust. J. Agric. Res.* 1992; **43**: 935.
13. Hubick KT. *Aust. J. Plant Phys.* 1990; **17**: 413.
14. Livingston NJ, Guy RD, Sun ZJ, Ethier GJ. *Plant Cell Environ.* 1999; **22**: 282.
15. Ripullone F, Lauteri M, Grassi G, Amato M, Borghetti M. *Tree Physiol.* 2004; **24**: 671.
16. Brück H, Jureit C, Hermann M, Schulz A, Sattelmacher B. *Plant Biol.* 2001; **3**: 326.
17. Högberg P, Johannisson C. *Plant Soil* 1993; **157**: 147.
18. Poulton PR. *Guide to the Classical and Other Long-term Experiments, Datasets and Sample Archive*, Lawes Agricultural Trust Co. Ltd., 2006; 51.
19. Available: http://www.res.bbsrc.ac.uk/era/.
20. Johnston AE, Garner HV. *Broadbalk Historical Introduction. Rothamsted Experimental Station. Report for 1968*, part 2; 1.
21. Kerley SJ, Jarvis SC. *Plant Soil* 1996; **178**: 287.
22. Zhao BZ, Maeda M, Ozaki Y. *Soil Sci. Plant Nutr.* 2002; **48**: 555.
23. Choi WJ, Ro HM, Hobbie EA. *Soil Biol. Biochem.* 2003; **35**: 1493.
24. Choi WJ, Ro HM, Lee SM. *Soil Biol. Biochem.* 2003; **35**: 1289.
25. Watzka M, Buchgraber K, Wanek W. *Soil Biol. Biochem.* 2006; **38**: 1564.
26. Goulding KWT, Poulton PR, Webster CP, Howe MT. *S.U.M.* 2000; 244.
27. Högberg P. *Oecologia* 1990; **84**: 229.
28. Bol R, Eriksen J, Smith P, Garnett MH, Coleman K, Christensen BT. *Rapid Commun. Mass Spectrom.* 2005; **19**: 3216.
29. Riga A, van Praag HJ, Brigode N. *Geoderma* 1971; **6**: 213.
30. Gerzabek MH, Haberhauer G, Kirchmann H. *Soil Sci. Am. J.* 2001; **65**: 352.
31. Gerzabek MH, Pichlmayer F, Kirchmann H, Haberhauer G. *Eur. J. Soil Sci.* 1997; **48**: 273.
32. Choi W, Lee SM, Ro HM, Kim KC, Yoo SH. *Plant Soil* 2001; **245**: 223.
33. Farquhar GD, Richards RA. *Aust. J. Plant Physiol.* 1984; **11**: 539.
34. Brück H, Jureit C, Hermann M, Schulz A, Sattelmacher B. *Plant Biol.* 2001; **3**: 326.
35. Asch F, Dingkuhn M, Dorffling K. *Plant Soil* 2000; **218**: 1.
36. Clay DE, Engel DS, Long DS, Liu Z. *Soil Sci. Soc. Am. J.* 2001; **65**: 1823.
37. Rebetzke GT, Richards RA, Condon AG, Farquhar GD. *Euphytica* 2006; **150**: 97.

DOI: 10.1002/rcm