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Trends in $^{13}C/^{12}C$ ratios and C isotope discrimination of wheat since 1845

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Abstract Archived wheat grain and straw samples from the unfertilised plot of the Broadbalk Continuous Wheat Experiment at Rothamsted, England, were used to investigate the trends in stable C isotope ratios since 1845. Grain $\delta^{13}C$ was higher than straw $\delta^{13}C$. Both grain and straw $\delta^{13}C$ have decreased by approximately 2.5–2.8‰ over the last 153 years, and since the 1960s the decrease has been more rapid than the decrease of the $\delta^{13}C$ of atmospheric CO_2. The C isotope discrimination (Δ), and hence the ratio of CO_2 concentration in leaf intercellular space to that in the ambient air (c_i/c_a), remained relatively stable or decreased slightly between 1845 and the mid-1960s, then increased considerably. The period with increasing Δ and c_i/c_a corresponds to the introduction of the modern short-straw cultivars of wheat. When grown on the unfertilised plot in the same seasons, a modern wheat cultivar had slightly higher Δ values than an old cultivar. The c_i derived from both grain and straw Δ have increased by 33–37% over the last 153 years, with the increase being more rapid after than before the mid-1960s. However, there was no clear trend in the yields of straw and grain, primarily because of the limitations of N and other nutrients, and also because of large year-to-year variations.

Keywords Atmospheric CO_2 · Biomass · Carbon isotope ratio · Long-term trend · Wheat

Introduction

The concentration of atmospheric CO_2 has increased from approximately 285 to 355 μmol mol^{-1} during the last 150 years as a result of anthropogenic emissions and deforestation. Short-term CO_2 enrichment experiments have demonstrated that increasing CO_2 concentration stimulates plant photosynthesis and productivity (Lawlor and Mitchell 1991; Polley et al. 1993; Beerling and Woodward 1995, and references therein). Global C models have also predicted increased C sequestration by terrestrial biosphere (Keeling et al. 1996; Cao and Woodlard 1998). However, direct evidence of increased plant productivity in terrestrial ecosystems has been more difficult to obtain, and substantial controversy still exists (LaMarche et al. 1984; Kienast and Luxmoore 1988; Woodward 1993). For example, Jenkinson et al. (1994) found no significant trends in the herbage yields over the last century in the Rothamsted Park Grass experiment, and concluded that it was very difficult to establish small long-term yield trends because of large year-to-year variation in biomass production.

Associated with the increasing concentration of atmospheric CO_2 is a decreasing trend of its $^{13}C/^{12}C$ ratio ($\delta^{13}C$), which has decreased from about –6.5 to –8.0‰ over the last 150 years (Friedli et al. 1986; Keeling et al. 1989; Fig. 1). Long-term decline in $\delta^{13}C$ has been reported in herbarium and tree-ring samples (Stuiver et al. 1984; Leavitt and Long 1988; Peñuelas and Azcón-Bieto 1992; Woodward 1993; Feng 1998; Tang et al. 1999). However, these records have the disadvantages of either unknown locations and lack of the information for environmental conditions in the case of herbarium samples, or complications of the juvenile effect in the case of tree-ring samples (Francey and Farquhar 1982; Duquesnay et al. 1998).

The $^{13}C/^{12}C$ ratio in plants is a powerful tool that allows estimation of leaf-gas exchange and water-use characteristics integrated over the entire growth period (Farquhar et al. 1989). Applying this approach to herbarium and tree-ring samples, several researchers have shown that both the integrated concentration of CO_2 in leaf intercellular space (c_i) and intrinsic water-use efficiency of plants have increased considerably over the last 1–2 centuries (Peñuelas and Azcón-Bieto 1992;

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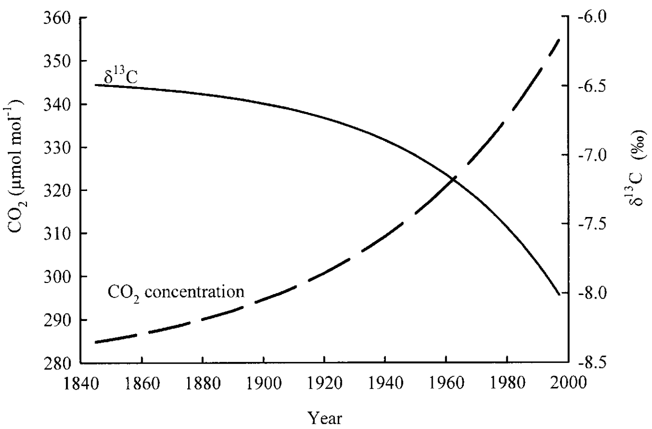

Fig. 1 Trends in the concentration and $\delta^{13}C$ of atmospheric CO_2 (based on the regression equations given by Feng (1998)

Woodward 1993; Feng 1998; Tang et al. 1999). These changes imply increased potential C assimilation in plants.

In the present study, we analysed C stable isotope ratios in a unique archive of wheat straw and grain samples, dating back to 1845. The samples are from the world's oldest field experiment, the Broadbalk Continuous Wheat Experiment at Rothamsted, southeast England, which has been run continuously since 1843. We further estimated the trends in the C isotope discrimination (Δ) and c_i .

Materials and methods

The Broadbalk Continuous Wheat Experiment

The Broadbalk Experiment at Rothamsted started in 1843 in a semi-rural environment in Hertfordshire, England, approximately 40 km north of London (longitude 0° 21′ W, latitude 51° 49′ N, elevation 128 m). The soil belongs to the moderately well drained Batcomb series (Aquic Paleudalf) with a flinty silty clay loam textured topsoil on clay-with-flints overlying a chalky parent material. The original objective of the experiment was to test the effects of different inorganic and organic fertilisers on wheat yield. However, the experiment and the associated large array of archived crop and soil samples have become a unique and invaluable resource for a wide range of agricultural, environmental and ecological studies (Johnston 1997). The experiment occupies approximately 5 ha of land, and was originally divided into 20 parallel plots (0.24 ha each) for different treatments of inorganic and organic fertilisers. Because the experiment was started well before the advent of modern statistics, treatments were not replicated. In the late nineteenth century, 1.5-m-wide paths between each plot were introduced, reducing the actual plot size to 0.19 ha. Subsequently the plots were subdivided transversely into sections, each of which received different agronomic treatments (crop rotation, straw incorporation, herbicide, fungicide and insecticide), but the same fertiliser treatment. Two sections of each plot have been maintained for growing wheat (*Triticum aestivum*) continuously since 1843, apart from the 1926–1951 period, when they remained fallow for 1–5 years to control weeds. The cultivars grown in the experiment were the common ones in the United Kingdom during different periods; these are listed in Table 1. Winter wheat is normally sown in October and harvested in August, and yields of grain and straw determined. Sub-samples of straw and grain were dried at 80°C for 16 h before being archived in sealed glass jars or tin boxes. For more details of the experiment, see the web pages of the Electronic Rothamsted Archive (http://www.res.bbsrc.ac.uk/era/) and Johnston and Garner (1969).

In this study, we chose 38 straw and 45 grain samples from a continuous wheat section (section 1) of the control plot (plot 3), which has not received any fertilisers since 1843. Samples were selected in approximately 5-year intervals, starting from 1845. In addition, the yearly samples between 1970 and 1978 and between 1990 and 1997 were also included. Most of the grain and straw samples were from the same year, except in a few cases when either straw or grain samples were missing from the archive and samples from the next available year were used.

Between 1987 and 1990, an experiment was carried out to compare biomass production and the response to N fertiliser of an old long-straw cultivar (Square Head's Master) and a modern short-straw cultivar (Brimstone) of winter wheat (Austin et al. 1993). The two cultivars were grown side by side in selected plots of the Broadbalk experiment. Square Head's Master is a land race, which was commonly grown in England during the nineteenth and early twentieth centuries. It was grown on the Broadbalk experiment for 39 seasons during 1900–1967 (Table 1). Brimstone was introduced into agriculture in 1985, and grown on Broadbalk during 1985–1990. Grain and straw samples of the two cultivars grown on the control plot were used to investigate whether the old and modern cultivars differ in the $^{13}C/^{12}C$ ratio when grown under the same conditions.

Isotopic analysis

Plant samples were dried at 80°C for 16 h, and ground to a homogenised fine powder. The $^{13}C/^{12}C$ ratio was determined in duplicate using dual inlet isotope ratio mass spectrometry (VG Optima). Samples were placed in tin capsules, combusted in an elemental analyser (Carlo Erba NA 1500) and purified in the on-line

Table 1 Wheat cultivars grown in the Broadbalk Experiment during different periods

Period	Cultivar	Period	Cultivar
1844–1848	Old Red Lammas	1930–1939	Red Standard
1849–1852	Old Red Cluster	1940–1941	Square Head's Master
1853–1881	Red Rostock	1942	Stand up
1882–1899	Red Club	1943	Square Head's Master
1900–1904	Square Head's Master	1944–1945	Red Standard
1905	Giant Red	1946–1967	Square Head's Master
1906–1909	Square Head's Master	1968–1978	Cappelle
1910	Bronwick Red	1979–1984	Flanders
1911–1912	Little Joss	1985–1990	Brimstone
1913–1916	Square Head's Master	1991–1995	Apollo
1917–1928	Red Stardard	1996–1997	Hereward
1929	Square Head's Master		

Triple Trap device prior to isotope ratio determination. Results are expressed as $\delta^{13}C$ in ‰ with respect to the PDB standard. The overall analytical reproducibility is 0.1‰.

Climatic data

Rothamsted has one of the longest continuous sets of weather recordings in the world, with rainfall and air temperature data available since 1853 and 1878, respectively. Rainfall is measured with a 1/1,000 acre gauge (4.047 m^2), which correlates closely with the measurements using the standard 5 inch (12.7 cm) gauge of the UK Meteorological Office but is on average 6.8% higher than the latter. Other climatic data used in this study were sunshine hours (data available since 1892) and potential soil moisture deficit (PSMD, data available since 1960). PSMD was calculated cumulatively from the first day of each year as the difference between evaporation over grass and daily rainfall. All climatic data were extracted from the Electronic Rothamsted Archive (http://www.res.bbsrc.ac.uk/era/).

Calculations

Stable C isotope ratio of plant samples is expressed in the standard δ notation:

$$\delta^{13}C_{\text{sample}} = \left[\frac{(^{13}C/^{12}C)_{\text{sample}}}{(^{13}C/^{12}C)_{\text{standard}}} - 1\right] \times 1000 \quad (1)$$

where the standard is the carbon dioxide generated from the PDB fossil limestone.

For C3 plants, the relationship between C isotope discrimination and leaf gas exchange can be described by the following equation (Farquhar et al. 1989):

$$\Delta = a + (b - a)c_i / c_a \quad (2)$$

where Δ is the C isotope discrimination in plants:

$$\Delta = \frac{\delta^{13}C_{\text{air}} - \delta^{13}C_{\text{plant}}}{1 + \delta^{13}C_{\text{plant}} / 1000} \quad (3)$$

and a is the discrimination against $^{13}CO_2$ due to diffusion of CO_2 in free air (=4.4‰), b is the fractionation associated with RuBP carboxylase (~27‰), and c_i and c_a are the concentrations (μmol mol^{-1}) of CO_2 in the leaf intercellular space and in the atmosphere, respectively. To calculate Δ using Eq. 3 requires knowledge of the variation of C isotope ratio in the atmosphere ($\delta^{13}C_{air}$). The $\delta^{13}C_{air}$ for different years was estimated from the following regression equation given by Feng (1998):

$$\delta^{13}C_{\text{air}} = -6.429 - 0.0060 \exp[0.0217(t - 1740)] \quad (4)$$

where t is calendar year. Equation 4 was derived from direct measurements of the $\delta^{13}C$ of the atmospheric CO_2 (Keeling et al. 1989) and of CO_2 separated from air bubbles in polar ice cores (Friedli et al. 1986) (Fig. 1). It was assumed that the $\delta^{13}C_{air}$ at the experimental site equalled the $\delta^{13}C_{air}$ of the ambient atmosphere.

Knowing Δ, c_i/c_a can be calculated by rearranging Eq. 2:

$$\frac{c_i}{c_a} = \frac{\Delta - a}{b - a} \quad (5)$$

The concentration of atmospheric CO_2 has increased exponentially during the last 2 centuries. Feng (1998) gave the following regression equation to describe the trend of c_a with time (Fig. 1):

$$c_a = 277.78 + 1.350 \exp[0.01572(t - 1740)] \quad (6)$$

From Eqs. 5 and 6, c_i can be calculated.

Results

Climatic and crop yield data

Annual rainfall varied between 409 and 983 mm during the 1853–1997 period, with a mean of 724 mm, but there was no significant trend (Fig. 2). In contrast, annual means of daily maximum temperature, and particularly, daily minimum temperature increased significantly (P<0.05 and 0.001, respectively) during the 1878–1997 period (Fig. 2). The equations obtained from linear regression showed that annual means of daily maximum and minimum temperatures increased by 0.48°C and 0.95°C, respectively, over the last 120 years.

Over the period from 1843 to 1997, the mean dry matter yields of grain and straw of the unfertilised control plot (the continuous wheat section; ± standard deviation) were 1.04±0.40 and 1.34±0.80 t ha^{-1}, respectively. There was a fundamental change in the pattern of biomass distribution between straw and grain after 1968, when modern short-straw cultivars were introduced, which produced more grain than straw (Fig. 3). The biomass data were highly variable between years, partly due to changes in the harvesting methods over the last 150 years, but in general there were no clear trends in ei-

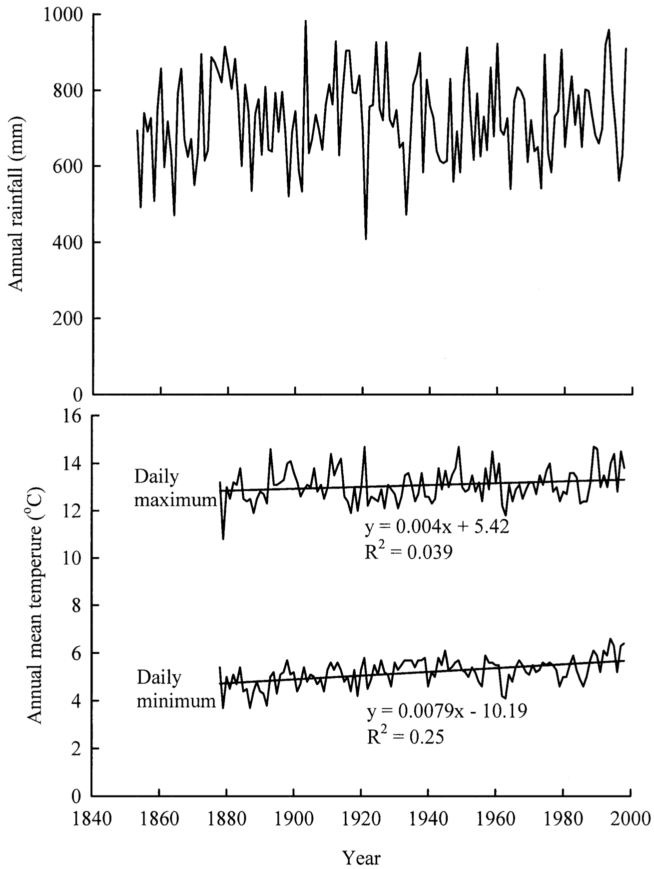

Fig. 2 Trends in annual rainfall and annual means of daily maximum and minimum temperatures at Rothamsted

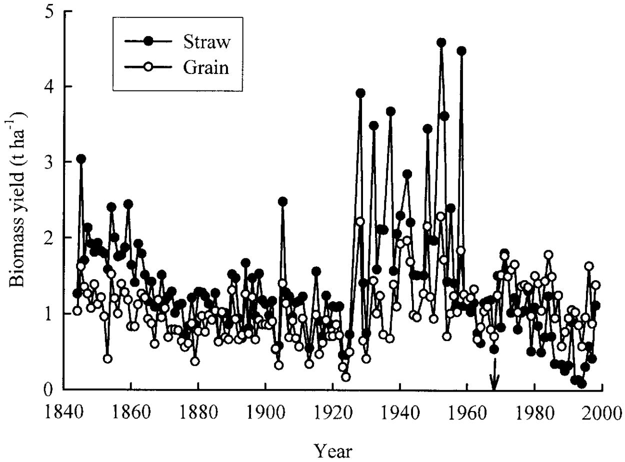

Fig. 3 Yields of grain and straw dry matter in the unfertilised plot of the Broadbalk experiment. *Arrow* indicates the change from long-straw to short-short cultivars

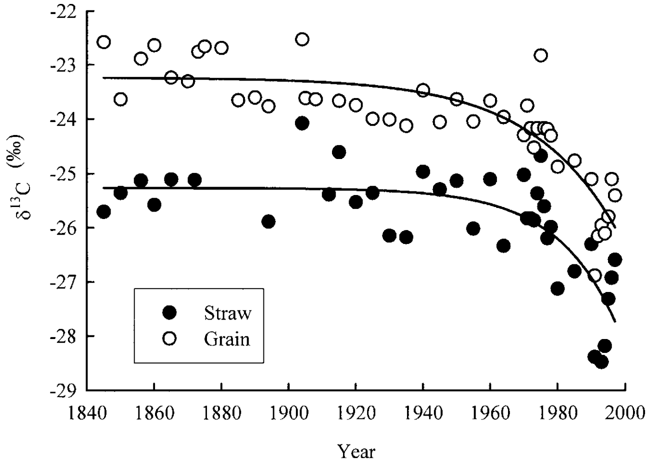

Fig. 4 Trends in $\delta^{13}C$ of wheat grain and straw from the unfertilised plot of the Broadbalk experiment

Table 2 $\delta^{13}C$ (‰) of an old cultivar (Square Head's Master) and a modern cultivar (Brimstone) of wheat grown side by side on the control plot of the Broadbalk experiment. *NA* Sample not available

Year	Grain		Straw	
	Square Head's Master	Brimstone	Square Head's Master	Brimstone
1987	–24.95	–25.53	NA	NA
1988	–25.89	–25.67	–26.85	–26.78
1989	–24.20	–24.78	–26.00	–26.34
1990	–24.96	–25.38	–26.23	–26.77
Mean	–25.00	–25.34	–26.36	–26.63

ther straw or grain yields in the pre- or post-1968 periods.

Comparison between old and modern wheat cultivars in $\delta^{13}C$

When grown side by side on the control plot of the Broadbalk experiment, the modern cultivar Brimstone had a slightly lower $\delta^{13}C$ in both grain and straw than the old cultivar Square Head's Master (Table 2). On average of the four growing seasons (three for straw), the difference in $\delta^{13}C$ between the two cultivars was about 0.3‰. The $\delta^{13}C$ of grain was always higher than that of straw in both cultivars. The results indicate that the C isotope discrimination (Δ) of the modern cultivar Brimstone was slightly larger than the old cultivar Square Head's Master.

Trends in $\delta^{13}C$ of wheat grain and straw

Both grain and straw samples showed a clear decreasing trend in $\delta^{13}C$, particularly after 1960 (Fig. 4). Similar to the trend in $\delta^{13}C$ of atmospheric CO_2 (see Feng 1998), the time series of wheat grain and straw $\delta^{13}C$ can be fitted with exponential curves (Fig. 4):

for grain:

$$\delta^{13}C = -23.24 - 0.00605 \exp[0.0398(t-1843)],\quad R^2 = 0.74 \tag{7}$$

and

for straw:

$$\delta^{13}C = -25.27 - 0.00038 \exp[0.0571(t-1843)],\quad R^2 = 0.59 \tag{8}$$

According to the above exponential curves, grain and straw $\delta^{13}C$ decreased by 2.75‰ and 2.50‰, respectively, over the 153 years from 1845 to 1997. These compared to a decrease of 1.53‰ in the $\delta^{13}C$ of atmospheric CO_2 during the same period estimated from Eq. 4. The decrease in grain and straw $\delta^{13}C$ appeared to be more rapid than the decrease in the $\delta^{13}C$ of atmospheric CO_2 during the 1960s to 1990s. Grain and straw $\delta^{13}C$ correlated closely (r=0.87, P<0.001), but on average, the former was 1.9‰ higher than the latter.

Trends in Δ, c_i/c_a and c_i

To derive the long-term trend in plant C isotope discrimination (Δ), the trends extracted from the plant $\delta^{13}C$ data, represented by Eqs. 7 and 8, and the trend of $\delta^{13}C$ of atmospheric CO_2 (Eq. 4; Fig. 1) were substituted into Eq. 3. This approach is considered to be effective for filtering out the high-frequency noise while preserving the low-frequency signals that carry relevant long-term trend (Feng 1998). The trend of Δ appears to have two distinctive phases (Fig. 5). Between 1845 and mid 1960s, Δ for grain was relatively constant, and for straw decreased slightly (by 0.35‰). Since the mid-1960s, both straw and grain Δ have increased considerably (by 1.4‰).

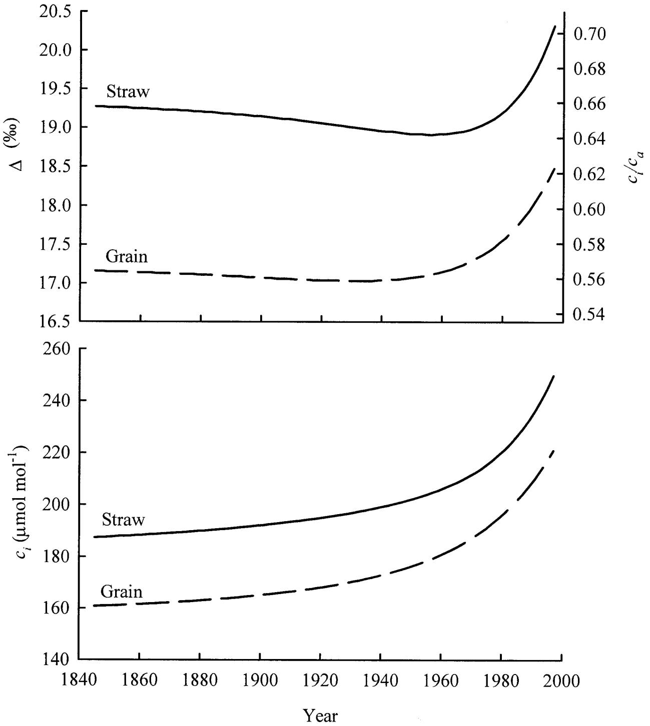

Fig. 5 Trends in Δ, c_i/c_a and c_i for the wheat grain and straw samples from the unfertilised plot of the Broadbalk experiment

The patterns of c_i/c_a (Fig. 5) were exactly the same as those of Δ, because of the linear relationship between the two. The c_i/c_a derived from the straw isotopic data was considerably higher than that for grain, because the former had larger Δ. Between 1845 and the mid-1960s, c_i/c_a showed little change in grain and a small decrease in straw. Since the mid-1960s, c_i/c_a has increased by about 0.06 (approximately 10% increase).

The c_i values for both straw and grain (Fig. 5) increased by 33–37% as atmospheric CO_2 (Fig. 1) increased by approximately 25% between 1845 and 1997. The increase in c_i was more rapid over the last 35 years than over the first 115 years.

Correlation between Δ and climatic data

To examine the effect of climatic variations on plant Δ, grain and straw Δ were calculated from their respective $\delta^{13}C$ and the $\delta^{13}C$ of atmospheric CO_2 of the corresponding year. Grain and straw Δ correlated poorly with all of the climatic variables examined (rainfall, PSMD, temperature and sunshine). The only correlation that nearly reached the significance level of P<0.05 was between straw Δ and the total rainfall between April and July, the active growing period for wheat crops (Fig. 6).

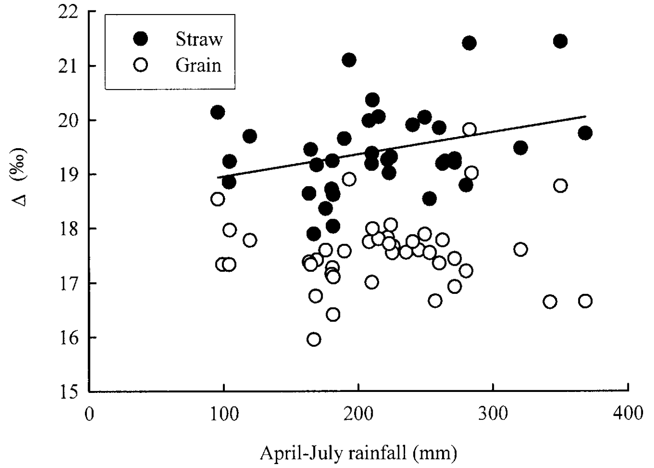

Fig. 6 Relationships between grain and straw Δ and April-July rainfall (regression shown for straw only, y=18.54+0.0041x, R^2=0.10, P=0.055)

Discussion

Marino and McElroy (1991) demonstrated that $\delta^{13}C$ of C4 plants can be used as a proxy for the $\delta^{13}C$ of atmospheric CO_2, because their $\delta^{13}C$ varies little with environmental conditions. They showed a clear decreasing trend in the $\delta^{13}C$ of maize kernels grown at the same location from 1948 to 1986. For C3 plants like wheat, $\delta^{13}C$ varies much more widely as a result of environmental variations, and it would be impossible to derive $\delta^{13}C$ of atmospheric CO_2 in the past accurately from stored C3 plant samples. Even so, the decline in the $\delta^{13}C$ of atmospheric CO_2 over the last 150 years was clearly reflected in the $\delta^{13}C$ of wheat grain and straw samples archived from the Broadbalk experiment, both having decreased in an exponential fashion. It is also clear that the $\delta^{13}C$ of the wheat samples has decreased faster than the $\delta^{13}C$ of atmospheric CO_2 since the mid-1960s. Previously, long-term time series of plant $\delta^{13}C$ have only been reported in tree-ring or herbarium samples (Stuiver et al. 1984; Leavitt and Long 1988; Peñuelas and Azcón-Bieto 1992; Woodward 1993; Duquesnay et al. 1998; Feng 1998; Tang et al. 1999). The present study has added a valuable long-term data set for an annual crop grown continuously on the same plot, and allowed an analysis of the effects of climate and crop yield.

In the calculation of Δ, the trend of decreasing $\delta^{13}C$ of atmospheric CO_2 has been removed. Variation of Δ in plants is caused by both genetic variation and the effects of environmental factors on plant physiological and biochemical processes. Of the environmental factors that influence plant Δ, water availability is usually the most noticeable (Farquhar et al. 1989). In this study, however, straw Δ of the control plot over the last 153 years correlated only weakly with rainfall during the active growing period, and grain Δ did not correlate with any of the climatic variables examined. This is likely to be due to the overriding limitation of N in this unfertilised plot, with the associated small crop canopy and infrequent water stress.

Δ and c_i/c_a remained stable or decreased slightly between 1845 and the mid-1960s, but has shown a rapid increase since the mid-1960s. The second phase appeared to correspond to the introduction of the modern short-straw cultivars of wheat, as a result of the "green revolution". When grown under the same conditions at the same time, the modern cultivar Brimstone had a higher Δ than the old cultivar Square Head's Mater, although the difference was not large. The difference may imply an increased stomatal conductance in the modern cultivars, which in turn may lead to a higher biomass potential if water and nutrients are not limiting (Condon et al. 1987). To establish whether old and modern cultivars have a systematic difference in C isotope discrimination requires direct comparisons under the same growing conditions of more cultivars than were available in our studies.

Apart from the cultivar factor, there are several possible explanations for the increasing trend in plant Δ and c_i/c_a. First, N supply in this unfertilised plot may have been depleted gradually because of annual removal of straw and grain, resulting in a gradual decrease of photosynthetic capacity in leaves, and hence an increase of c_i/c_a and Δ. Such response may be greater with the modern cultivars than the old ones, because biomass and grain yields of the modern cultivars are much more responsive to N supply than the old cultivars (Austin et al. 1993). However, against this argument is the observation that atmospheric N deposition to the experimental site has increased from about 10 kg ha^{-1} in 1843 to 45 kg ha^{-1} today (Goulding et al. 1998) and N removal in the crop has remained approximately constant over the entire period. Second, the increasing trend of Δ and c_i/c_a since the mid-1960s may be a result of increasing atmospheric CO_2. Using a growth chamber experiment, Polley et al. (1993) found that c_i/c_a of wheat increased by about 0.03 (P<0.05) when CO_2 increased from 225 to 350 µmol mol^{-1}. It must be pointed out, however, that not all species respond to increasing CO_2 in the same way. Beerling and Woodward (1995) reported small increases of c_i/c_a and Δ with increasing CO_2 in 17 C3 temperature grass and herb species in a growth chamber experiment, whereas Feng (1998) showed that the long-term trends in c_i/c_a derived from tree-ring δ^{13}C chronologies could be either positive, negative or rather constant. Thirdly, the long-term trend of grain Δ and c_i/c_a could be due to long-term climatic changes, in particular the increase in temperature (Fig. 2). The effects of temperature on Δ and c_i/c_a are complex and not well understood, and Feng (1998) concluded that the overall effect of rising temperature would be small.

In agreement with other studies (Farquhar and Richards 1984; Hubick and Gibson 1993), grain Δ was consistently smaller than straw Δ. The difference has been attributed to the difference in chemical composition between tissues (Farquhar and Richards 1984). Wheat grain contains more proteins than straw. On average, the grain samples used in this study contained 1.7% total N, corresponding to a total crude protein concentration of 9.7%, whereas the straw samples had an averaged total N of only 0.4%. The formation of C skeletons for some amino acids in proteins involves phospho-*enol*pyruvate carboxylation, which discriminates in favour of ^{13}C, resulting in higher δ^{13}C (lower Δ) in the total amino acid pool than bulk tissue (Farquhar and Richards 1984). In contrast, straw has a significant proportion (~15%) of lignin, and the δ^{13}C of lignin C is usually 5–6‰ lower than bulk tissue (Boutton 1996). Lipids are a minor constituent (<2%) of wheat grain (Hoseney 1986), and therefore are unlikely to be an important reason for the difference between grain and straw δ^{13}C. Alternatively, photosynthesis during the later phase of crop growth, when water shortage is more likely to occur, probably contributes more to the C in grain than in straw, resulting in higher δ^{13}C (lower Δ) in grain than in straw. This explanation is probably less important than the first in light of the poor correlation between grain Δ and climatic data in this study. A third explanation is that remobilisation of C from vegetative tissues to grain could possibly result in C isotope fractionation.

In accordance with previous reports from tree-ring studies (Feng 1998; Tang et al. 1999), the concentrations of CO_2 in leaf intercellular space (c_i) calculated from either straw or grain Δ have increased significantly during the past 153 years as the concentration of atmospheric CO_2 increased by about 25%. Theoretically, and also from empirical evidence, increases in c_i are expected to result in increases in C assimilation, provided that other growing conditions are not limiting (Polley et al. 1993; Beerling and Woodward 1995). This is not the case in the unfertilised plot of the Broadbalk experiment, because of the overriding limitation of N, and possibly also other nutrients. Additionally, the large year-to-year variation in crop biomass makes any long-term trend in biomass production difficult to detect.

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