

Intrinsic water-use efficiency of temperate seminatural grassland has increased since 1857: an analysis of carbon isotope discrimination of herbage from the Park Grass Experiment

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Abstract

A 150-year-long record of intrinsic water-use efficiency (W_i) was derived from community-level carbon isotope discrimination ($^{13}\Delta$) in the herbage of the unfertilized, unlimed control treatment (plot 3) of the Park Grass Experiment at Rothamsted (England) between 1857 and 2007. $^{13}\Delta$ during spring growth (first cut harvested in June) averaged 21.0‰ ($\pm 0.5\%$ SD) and has not shown a long-term trend ($P = 0.5$) since 1857. $^{13}\Delta$ of summer/autumn growth (second cut harvested between September and November) increased from 21.3‰ to 22.0‰ ($P < 0.001$) between 1875 and 2007. W_i during spring growth has therefore increased by 33% since the beginning of the experiment, and W_i of summer/autumn growth has increased by 18%. The variation in $^{13}\Delta$ was mainly related to weather conditions. Plant available soil water explained 51% and 40% of the variation in spring growth $^{13}\Delta$ and summer/autumn growth $^{13}\Delta$, respectively. In the 1857–2007 period yields have not increased, suggesting that community-level photosynthesis has not increased either. Therefore, the increased W_i probably resulted from a decreased stomatal conductance. Vapour pressure deficit (VPD) during spring growth (March–June) has not changed since 1915, meaning that instantaneous water-use efficiency (W_i) in spring time has increased and transpiration has probably decreased, provided that leaf temperature followed air temperature. Conversely, VPD in the months between the first and second cut has increased by 0.07 kPa since 1915, offsetting the effect of increased W_i on W_t during summer and early autumn. Our results suggest that vegetation has adjusted physiologically to elevated CO_2 by decreasing stomatal conductance in this nutrient-limited grassland.

Keywords: C-13, C₃ grassland, climate change, CO₂, herbage, long-term trend, transpiration, water-use efficiency

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Introduction

Terrestrial ecosystems play an important role in the global carbon and water cycle. For the prediction of future climate change, it is necessary to understand how ecosystems respond to rising CO₂ concentrations. Multiyear free-air CO₂ enrichment (FACE) experiments with elevated CO₂ (Ainsworth & Rogers, 2007) indicate that C₃ grasses decrease stomatal conductance by over 30% and increase light-saturated CO₂ uptake by over 30% when growing under elevated (567 $\mu\text{mol mol}^{-1}$, average of all studies) as compared with ambient CO₂ concentrations (366 $\mu\text{mol mol}^{-1}$, average of all studies).

Accordingly, one would expect that the water-use efficiency of grasslands has also increased during the CO₂ increase of the Anthropocene. But, this has not been verified in detail. Results from short-term experiments show the complexity of grassland responses to rising CO₂ and their interactions with other environmental parameters: Shaw *et al.* (2002) showed that elevated CO₂ suppressed the response of net primary production to increased temperature, precipitation and nitrogen availability in a California annual grassland. An increase of net primary production under elevated CO₂ was only observed when other environmental parameters remained unchanged. For an alpine grassland in Switzerland, Körner *et al.* (1997) found low responsiveness to elevated CO₂ and concluded that this ecosystem was

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already carbon saturated. In *Lolium perenne* L. swards, Schneider *et al.* (2004) showed that the responsiveness to elevated CO₂ was greater at high than at low nitrogen availability. However, as acclimation responses and feedback mechanisms may occur in the long term, it is difficult to extrapolate from the relatively short-term responses of FACE experiments to long-term ecosystem responses.

Retrospective analysis of carbon isotope discrimination (¹³Δ) is one way to assess the effect of CO₂ on the biological coupling of carbon and water cycles over past decades and even centuries. According to the linear form of the Farquhar carbon isotope discrimination model, ¹³Δ depends on the relationship between assimilation (*A*) and stomatal conductance (*g_s*) which determine the ratio of intercellular (*c_i*) to atmospheric (*c_a*) CO₂ concentration (*c_i/c_a*)

$$^{13}\Delta = a + (b - a) \frac{c_i}{c_a}, \quad (1)$$

with *a* denoting the fractionation that occurs during diffusion of CO₂ in air (4.4‰) and *b* the net fractionation due to carboxylation (27‰) (Farquhar *et al.*, 1989). Estimates of ¹³Δ are obtained from the carbon isotope composition of plant material (δ¹³C_p) and the atmosphere (δ¹³C_a)

$$^{13}\Delta = \frac{\delta^{13}\text{C}_a - \delta^{13}\text{C}_p}{1 + \delta^{13}\text{C}_p}. \quad (2)$$

(Farquhar *et al.*, 1989).

The relationship between *A* and *g_s* is termed leaf-level 'intrinsic' water-use efficiency (*W_i*) (Ehleringer *et al.*, 1993) and given by

$$W_i = \frac{A}{g_s} = \frac{c_a - c_i}{1.6} = \frac{c_a \left(1 - \frac{c_i}{c_a}\right)}{1.6}, \quad (3)$$

where 1.6 stands for the ratio of the diffusivities of water vapour and CO₂ in air. *W_i* is directly relevant to the comparison of water-use efficiencies among genotypes or species growing together in the field in the same environment. As is evident from Eqn (3), *W_i* can be estimated directly from knowledge of ¹³Δ [see Eqn (1)] and *c_a*, but it does not include the effect of the leaf-to-air difference in the mole fraction of water vapour [*v*, see Eqn (9)] on plant transpiration. The effect of *v* is accounted for by the 'instantaneous' water-use efficiency (*W_t*), which is defined as the ratio of *A* to transpiration (*E*) of leaves (Farquhar *et al.*, 1989)

$$W_t = \frac{A}{E} = \frac{c_a - c_i}{1.6v} = \frac{c_a \left(1 - \frac{c_i}{c_a}\right)}{1.6v}. \quad (4)$$

Thus, ¹³Δ and *W_i* [Eqn (3)] can be used to assess long-term trends in the balance between carbon gain and

intrinsic (or potential) water loss of plants, whereas estimates of *W_t* [Eqn (4)] also require chronologies of *v* to estimate (actual) instantaneous leaf-level water-use efficiency.

To date, long-term isotope studies on the response of *W_i* to rising atmospheric CO₂ concentrations and climate change were mainly done with trees, whose woody tissue and year rings provide convenient archives of stable carbon isotopes. Conversely, long-term series of ¹³Δ from herbaceous plants are rare and have been analysed in only a few studies (Peñuelas & Azcón-Bieto, 1992; Zhao *et al.*, 2001; Pedicino *et al.*, 2002). The response of plants to rising CO₂ concentrations can be analysed in terms of three scenarios: (i) constant *c_i/c_a*, (ii) constant *c_i*, or (iii) constant *c_a - c_i*, as discussed by Saurer *et al.* (2004). Trees did not show identical responses to rising CO₂ concentrations in terms of *c_i/c_a*, which remained constant, increased or decreased. However, *W_i* increased in most studies (Bert *et al.*, 1997; Duquesnay *et al.*, 1998; Feng, 1999; Arneeth *et al.*, 2002; Saurer *et al.*, 2004). This could result from decreasing stomatal conductance or increasing photosynthesis. Changes of assimilation might modify carbon storage in grassland soils, whereas changes in stomatal conductance and concurrent effects on evapotranspiration could influence water relations from the plant to the ecosystem and global scale. Decreasing stomatal conductance leads to warmer and drier air near the earth's surface (Betts *et al.*, 2000) and increases run-off (Betts *et al.*, 2007). It is unknown how grassland has responded to rising CO₂ concentration in the long-term and whether results from FACE experiments can be extrapolated to (semi)natural grassland ecosystems. Our aim was to assess the long-term response of grassland vegetation to rising CO₂ concentrations by analysing δ¹³C, ¹³Δ, *W_i*, and *W_t*, using archived vegetation samples from the Park Grass Experiment at Rothamsted, England.

The Park Grass Experiment is the world's oldest long-term ecological experiment and an almost perfect resource for stable isotope analysis: it offers the unique possibility of analysing archived herbage samples from temperate, seminatural grassland, dating back to 1857. The experiment was started in 1856 to test the effect of different amounts and combinations of inorganic fertilizers and organic manure on hay yield. We analysed samples from an unfertilized, unlimed treatment (plot 3). We chose this 'control plot' for our study, because its botanical composition, with regard to the proportion of grasses, legumes and forbs, has been in equilibrium for many years (Silvertown *et al.*, 2006). Thus, we minimized any influence of changing botanical composition on the community-level δ¹³C signal. Weather records (rainfall, temperature, sunshine, and wind data) were available for the experimental site from the electronic

Rothamsted Archive (eRA, 2008a). This made it possible to analyse both the long-term response to rising CO₂ concentrations and the short-term reaction of community-level $\delta^{13}\text{C}$ and derived parameters to year-to-year variation of weather conditions.

Our aims were to:

- (1) determine the long-term trends of carbon isotope composition ($\delta^{13}\text{C}$), carbon isotope discrimination ($^{13}\Delta$) and of intrinsic water-use efficiency (W_i) and instantaneous water-use efficiency (W_i),
- (2) assess the influence of interannually changing weather conditions on carbon isotope discrimination ($^{13}\Delta$) and
- (3) examine which environmental or meteorological parameter best predicts $^{13}\Delta$ in a seminatural grassland plant community.

Materials and methods

The Park Grass Experiment

The Park Grass Experiment was started in 1856 at the Rothamsted agricultural research station, located approximately 40 km north of London in Hertfordshire, England (0°21' West, 51°49' North, 128 m height above sea level). About 2.8 ha of old grassland were divided into 20 plots which received different fertilizer and manure treatments. The herbage was cut and made into hay, usually in mid-June. For the first 19 years of the experiment the regrowth was often grazed by sheep but, since 1875, a second cut has been taken, usually between late September and early November. Until 1960, samples of hay were taken from the material dried *in situ*. Since 1960 strips have been cut with a forage harvester and vegetation samples were taken directly from the fresh material and dried afterwards. However, the herbage on the rest of the plot was made into hay as before. The second cut has usually been cut and carted green. Samples from all plots have been dried and stored in the Rothamsted Archive since the beginning of the experiment (Silvertown *et al.*, 2006).

We analysed samples from one of the unfertilized, unlimed control plots (plot 3). In 1903, most plots were divided in two and lime was applied to one half every 4 years. The unlimed part of plot 3 was then named 3U. In 1965 the limed and unlimed subplots were divided again and three of the four subplots received chalk to achieve a pH of 5, 6, and 7, respectively. One subplot remained unlimed and this was named 3d (note: the whole experiment received a small amount of lime in the 1880s).

Studies of the botanical composition of the Park Grass plots have been made for 73 spring cut harvests by

separating harvested samples into guilds (grasses, legumes, other forbs) or even into single species to determine their percentage contribution to total harvested biomass. Since the beginning of the experiment, grasses have been dominant (mean/minimum/maximum: 60/29/91%) on plot 3 and on the unlimed subplots 3U and 3d, followed by other forbs (33/9/67%) while legumes contributed only a small percentage (7/0/15%). At the species level, the dominating grasses were *Agrostis capillaris* L. and *Festuca rubra* L., common forbs were *Centaurea nigra* L., *Leontodon hispidus* L. and *Plantago lanceolata* L. Data are available from the electronic Rothamsted Archive (eRA, 2008a).

The soil is a moderately well-drained silty clay loam overlying clay-with-flints and chalk. It is classified as stagnogleyic palaeo-argillic brown earth by the Soil Survey of England and Wales or as Aquic Paleudalf according to USDA classification (Avery & Catt, 1995). The water-holding capacity (or maximum plant available soil water, PAW_{capacity}) of the soil has not been measured directly, but was inferred from measurements on similar soils. It was 135 mm.

Sample preparation and isotope analysis

Representative subsamples of 2–3 g were taken from the archived samples of plot 3 (until 1902), half plot 3U (after 1903), and quarter plot 3d (after 1965). The first and the second cut were sampled and analysed separately. The subsamples were dried at 40 °C for 48 h, ball milled to a homogenous fine powder and dried again at 60 °C for 24 h. Aliquots of 0.7 ± 0.05 mg were weighed into tin cups (IVA Analysentechnik e.K., Meerbusch, Germany) and combusted in an elemental analyzer (NA 1110; Carlo Erba, Milan, Italy) interfaced (Conflo III; Finnigan MAT, Bremen, Germany) with an isotope ratio mass spectrometer (Delta Plus; Finnigan MAT). Carbon isotope data are presented in the conventional form as $\delta^{13}\text{C}$, where $\delta^{13}\text{C} = [(R_{\text{sample}}/R_{\text{standard}}) - 1]$, with R the $^{13}\text{C}/^{12}\text{C}$ ratio in the sample or standard (V-PDB). Each sample was measured against a laboratory working standard CO₂ gas, which was previously calibrated against a secondary isotope standard (IAEA-CH6 for ^{13}C , accuracy of calibration $\pm 0.06\%$ SD). After every 10th sample a solid internal laboratory standard (SILS) with similar C/N ratio as the respective sample material (fine ground wheat flour) was run as a control. The SILS were previously calibrated against an international standard (IAEA-CH6). The precision for sample repeats was better than 0.1‰ (SD of 57 SILS replications).

We tested if the change of the harvesting method in 1960 had an effect on the $\delta^{13}\text{C}$ of the vegetation samples. This analysis was possible as samples for the first cut in 1992, 1993, and 1994 had been obtained with both

harvesting methods. The comparison revealed that $\delta^{13}\text{C}$ of the samples taken from hay was less negative by 0.18‰ (± 0.06 SD). This phenomenon was probably related to larger losses of leaf material during hay making before 1960. To correct for this, we subtracted 0.18‰ from the $\delta^{13}\text{C}$ of the first cut samples collected before 1960.

Atmospheric CO_2 concentration and isotope composition

Atmospheric carbon isotope data ($\delta^{13}\text{C}_a$) and CO_2 concentrations were estimated following an approach by Wittmer *et al.* (2008). Data were taken from ice-core studies (Friedli *et al.*, 1986; Francey *et al.*, 1999; for $\delta^{13}\text{C}_a$ data before 1978 and for CO_2 concentration data before 1959) and from atmospheric monitoring (Mauna Loa: Keeling & Whorf, 2005; Shetland Islands: Allison *et al.*, 2003; Ocean Station M, Storhofdi Vestmannaeyjar Island, Mace Head: NOAA ESRL, 2007). Two cubic functions were fitted to the $\delta^{13}\text{C}_a$ data to estimate mean annual values. The first model ($\delta^{13}\text{C}_a = -26.5169 \cdot t^3 + 136.238 \cdot t^2 - 234.2816 \cdot t + 128.56$) provided estimates for the 1850–1958 period and the second model ($\delta^{13}\text{C}_a = 10693.7615 \cdot t^3 - 63649.6643 \cdot t^2 + 126245.672 \cdot t - 83450.9684$) for the 1959–2007 period, where t is the sampling year/1000. The cubic fit for the 1959–2007 period was forced to pass through the 1958 value estimated with the first model for the 1850–1958 period. The root mean squared error for the overall $\delta^{13}\text{C}_a$ model was 0.05‰. The $\delta^{13}\text{C}_a$ decreased from -6.5% in 1857 to -8.4% in 2007. For calculation of mean $\delta^{13}\text{C}_a$ during the two growing periods a seasonal correction factor was applied: analysis of monthly measured $\delta^{13}\text{C}_a$ (Allison *et al.*, 2003; NOAA ESRL, 2007) showed that, compared with the yearly average, $\delta^{13}\text{C}_a$ from March to June was more negative by 0.2‰ and $\delta^{13}\text{C}_a$ from July to November was more positive by 0.3‰.

A quadratic function was fitted to the CO_2 concentration data for the period from 1850 to 1958, and a cubic function for the period after 1959. The root mean squared error for the overall CO_2 concentration model was $0.6 \mu\text{mol mol}^{-1}$. CO_2 concentration increased from $287 \mu\text{mol mol}^{-1}$ in 1857 to $383 \mu\text{mol mol}^{-1}$ in 2007. Analysis of monthly measured CO_2 concentration data (Allison *et al.*, 2003; NOAA ESRL, 2007) showed that CO_2 concentration, compared with the yearly average, was higher during March to June ($+3.4 \mu\text{mol mol}^{-1}$) and lower during July to November ($-4.7 \mu\text{mol mol}^{-1}$).

Climatic data

Daily precipitation at Rothamsted has been measured since 1853 with a large rain gauge ('1/1000th acre gauge') covering 4.047 m^2 , measurements with a

Meteorological Office standard rain gauge are available since 1948. Rainfall data from both gauges correlated closely ($R^2 = 0.96$) but values from the 1/1000th acre gauge are on average 7% higher. We used data from the 1/1000th gauge, as it presents the longest continuous measurement. Where daily measurements were missing for calculation of PAW in the 1947–2007 period, they were estimated from the values measured with the standard gauge. Where this was not possible, a mean value of 3.6 mm day^{-1} rainfall was assumed (this was the case for 425 days after 1 January 1947, that is 2% of the data).

Temperature at Rothamsted has been measured daily since 1878. Daily mean temperature (\bar{T}_{Day}) was calculated according to Allen *et al.* (1998)

$$\bar{T}_{\text{Day}} = (T_{\text{Max}} + T_{\text{Min}})/2, \quad (5)$$

with T_{Min} the measured daily minimum and T_{Max} the measured daily maximum temperature.

The saturation vapour pressure (e) is related to air temperature (T) and can be derived using the equation given by Allen *et al.* (1998)

$$e(T) = 0.6108e^{\left(\frac{17.27T}{T+237.3}\right)}. \quad (6)$$

Daily mean saturation vapour pressure (e_s) was calculated as

$$e_s = \left(\frac{e(T_{\text{max}}) + e(T_{\text{min}})}{2}\right), \quad (7)$$

and actual vapour pressure (e_a) was calculated as the saturation vapour pressure at the dew point temperature (T_{Dew})

$$e_a = e(T_{\text{Dew}}). \quad (8)$$

Dew point temperature (T_{Dew}) was calculated from measured wet and dry bulb temperature (available since 1915) with the equation given in the electronic Rothamsted Archive (eRA, 2008b). The atmospheric vapour pressure deficit (VPD) was calculated as the difference of actual and saturation vapour pressure.

For calculation of W_t , the leaf-to-air difference in the mole fraction of water vapour is used, which is the VPD between the air and intercellular gas spaces of the leaf divided by total atmospheric pressure.

$$v = \frac{e_s - e_a}{p_{\text{total}}}, \quad (9)$$

where p_{total} is the total pressure of the atmosphere (99.8 kPa at 128 m height above sea level). It was assumed that leaf temperature was equal to mean air temperature.

Potential evapotranspiration (PET) was calculated using the FAO Penman–Monteith equation for a grass reference crop (Allen *et al.*, 1998). This equation

incorporates the effect of crop morpho-physiological characteristics on PET by using fixed coefficients for bulk surface resistance and aerodynamic resistance. PAW was calculated according to Schnyder *et al.* (2006)

$$PAW_i = PAW_{i-1} + P_i - AET_i, \quad (10)$$

with PAW_{i-1} the modelled PAW of the previous day, P_i the precipitation on day i , and AET_i the modelled actual evapotranspiration on day i . AET_i equalled PET_i as long as $PAW_i/PAW_{capacity} > 0.3$. Otherwise AET_i was calculated as

$$AET = \frac{PET}{0.3} \times \frac{PAW_i}{PAW_{capacity}}. \quad (11)$$

All weather parameters necessary for the calculation of PET, AET, and PAW were available for the 1947–2006 period or were derived from other parameters according to Allen *et al.* (1998): windspeed was calculated from windrun measurements, solar radiation was estimated from the measured duration of bright sunshine. Modelling was started on the 15th of February in 1947 when PAW_i was set to 135 mm (the PAW capacity), as the soil was most likely saturated with water at that time of the year.

Results

Local trends in climatic conditions

Mean annual rainfall was 735 mm (± 120 mm SD) between 1857 and 2007, and no significant long-term trend was observed. There was seasonality in the record, with highest rainfall in autumn (210 ± 68 mm) and lowest in spring (156 ± 50 mm), which is also reflected in other precipitation records from England and Wales (Wood, 2004). There was a peak in rainfall around 1875. Mean daily precipitation during spring (Fig. 1a) and summer/autumn (Fig. 1f) growth did not show significant trends.

Mean annual temperature has increased significantly since the beginning of the recording in 1878. But after 1990 the increase was more pronounced. Mean annual temperature in the 1878–1989 period was 9.1 °C, but in the 1990–2007 period it was 10.2 °C. Mean temperatures during spring (Fig. 1b) and summer/autumn (Fig. 1g) growth have increased significantly ($P < 0.0001$).

No trend was observed in the VPD during spring growth (Fig. 1c, $P = 0.4$). In contrast, the VPD in the summer/autumn period has increased by approximately 0.07 kPa since 1915 (Fig. 1h, $P < 0.01$). Thus, the annual sum of PET has increased significantly by

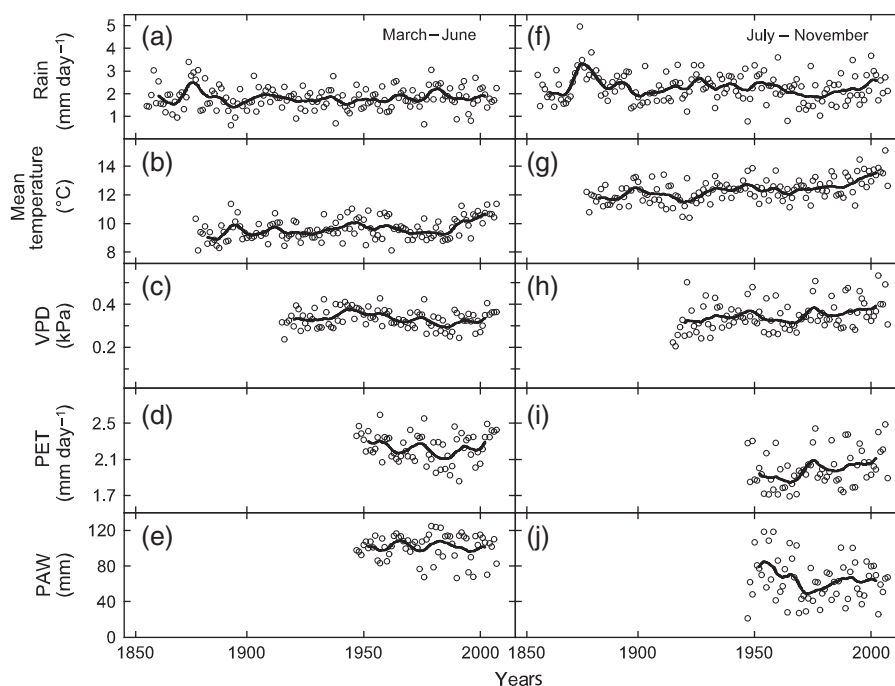


Fig. 1 Climate data (○) averaged over the growth season of the spring growth (March–June, left column) and the summer/autumn growth (July–November, right column). (a) and (f) mean daily rainfall, (b) and (g) mean temperature, (c) and (h) mean vapour pressure deficit (VPD), (d) and (i) mean potential evapotranspiration (PET), and (e) and (j) mean plant available soil water (PAW). Lines are the 11-years weighted moving averages. For analysis of trends best-fit linear regressions were calculated. Significant trends were observed for temperature which increased in both seasons ($P < 0.0001$) and for VPD and PET which increased in the summer/autumn season ($P < 0.01$ and $P < 0.05$).

0.66 mm yr⁻¹ ($P < 0.05$) from 603 mm in 1947 to 643 mm in 2007, but PET during spring growth showed no significant trend (Fig. 1d). Accordingly, no long-term change in PAW was observed in spring (Fig. 1e) and average PAW in June was 67 mm (± 33 mm SD). Mean daily PET during summer/autumn growth increased

significantly from 1.8 to 2.0 mm day⁻¹ from 1947 to 2007 (Fig. 1i, $P < 0.05$). However, PAW averaged from July to November did not show a significant trend during this period (Fig. 1j). Mean PAW in July was 45 mm (± 29 mm SD) and increased to 110 mm (± 33 mm SD) in November. Trends were evaluated using best-fit linear regression.

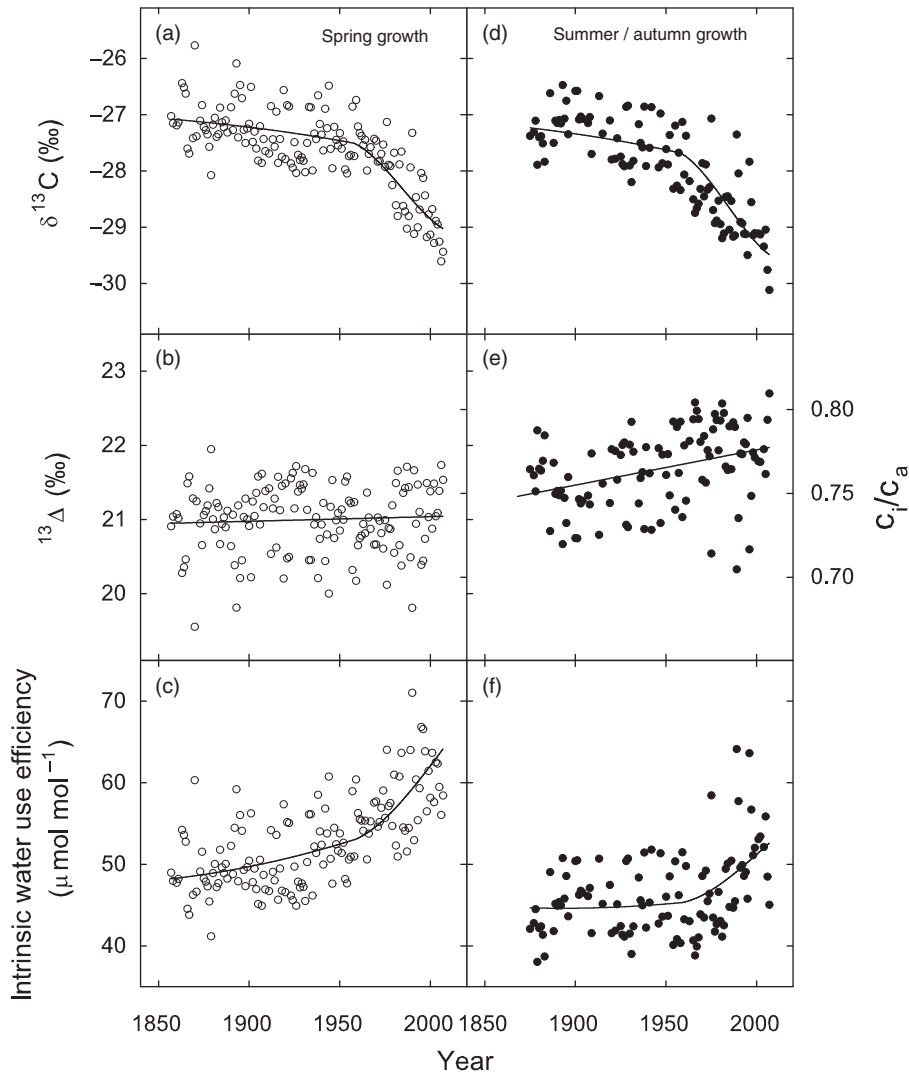


Fig. 2 Trends in carbon isotope composition ($\delta^{13}\text{C}$), carbon isotope discrimination ($^{13}\Delta$), the ratio of leaf internal to atmospheric CO_2 concentration (c_i/c_a) and intrinsic water-use efficiency (W_i). Graphs on the left side (a–c) show the trends for spring growth (○) since 1857, graphs on the right side (d–f) show the trends for summer/autumn growth (●) since 1875. Lines in (a) and (d) give the $\delta^{13}\text{C}_p$ values modelled from atmospheric $\delta^{13}\text{C}$, using the linear relationship between $\delta^{13}\text{C}_{\text{plant}}(y)$ and $\delta^{13}\text{C}_{\text{atm}}(x)$ (spring: $y = 1.05x - 20.1$, $R^2 = 0.63$, $P < 0.0001$; summer/autumn: $y = 1.25x - 19.4$, $R^2 = 0.67$, $P < 0.0001$). $^{13}\Delta$ was calculated from Eqn (2), lines in (b) and (e) are best-fit linear regressions, where $^{13}\Delta$ of summer/autumn growth is significant against year ($^{13}\Delta = 0.0048 \text{ year} + 12.4$, $P < 0.001$), while $^{13}\Delta$ of spring growth was not ($^{13}\Delta = 0.0006 \text{ year} + 19.8$, $P = 0.5$). W_i was calculated from Eqn (3): The line in (c) gives the modelled W_i values using Eqn (3) and assuming a constant mean c_i/c_a ratio of 0.73 for spring growth. The line in (f) gives the modelled W_i values calculated from Eqn (3) with modelled c_i/c_a derived from the linear regression of c_i/c_a against year ($c_i/c_a = 0.00021 \text{ year} + 0.355$, $P < 0.001$). W_i increased in proportion to c_a from 48 to 64 $\mu\text{mol mol}^{-1}$ (+ 33%) for the first cut and from 45 to 53 $\mu\text{mol mol}^{-1}$ (+ 18%) for the second cut.

Trends in $\delta^{13}\text{C}$ of vegetation

The $\delta^{13}\text{C}$ of the herbage ($\delta^{13}\text{C}_p$) decreased over the last 150 years, as did $\delta^{13}\text{C}_a$, with a more rapid decrease after 1960 (Fig. 2a and d). The linear relationship between $\delta^{13}\text{C}_p$ and $\delta^{13}\text{C}_a$ (data not shown) was used to model $\delta^{13}\text{C}_p$ without the interannual fluctuations from 1857 to 2007: thus $\delta^{13}\text{C}_p$ of the first cut decreased by 1.9‰ (from approximately -27.1‰ at $290\ \mu\text{mol mol}^{-1}$ to -29.0‰ at $386\ \mu\text{mol mol}^{-1}$, Fig. 2a), while $\delta^{13}\text{C}_p$ of the second cut decreased by 2.3‰ (from approximately -27.2‰ at $286\ \mu\text{mol mol}^{-1}$ to -29.5‰ at $378\ \mu\text{mol mol}^{-1}$, Fig. 2d) between 1875 and 2007.

Long-term trends in $^{13}\Delta$, c_i/c_a , c_i , and intrinsic water-use efficiency

There was no systematic long-term trend of $^{13}\Delta$ in the last 150 years for the first cut ($P = 0.5$) (Fig. 2b) and the long-term mean $^{13}\Delta$ was 21.0‰ ($\pm 0.5\text{‰}$ SD). $^{13}\Delta$ of the second cut increased by 0.7‰ from 21.3‰ in 1875 to 22.0‰ in 2007 (Fig. 2e, $P < 0.001$). As $^{13}\Delta$ and the c_i/c_a ratio are linearly related [Eqn (1)], the c_i/c_a ratio remained constant for the first cut (0.73 ± 0.02 SD) and increased slightly for the second cut from 0.75 to 0.78 (1875–2007). Accordingly, the intercellular CO_2 concentration increased with atmospheric CO_2 , rising from approximately 213 to $284\ \mu\text{mol mol}^{-1}$ for the first cut (1857–2007), and from approximately 214 to $294\ \mu\text{mol mol}^{-1}$ for the second cut (1875–2007). In the same way W_i increased from 48 to $64\ \mu\text{mol mol}^{-1}$ for spring growth (Fig. 2c) and from 45 to $53\ \mu\text{mol mol}^{-1}$ for the summer/autumn growth (Fig. 2f).

As the VPD remained constant from March to June in the long-term, W_t of spring growth increased in proportion to W_i , that is from 14.8 to $19.6\ \text{mmol mol}^{-1}$ in the 1915–2007 period (Fig. 3a). In contrast, the long-term increase of VPD in the months preceding the second cut compensated for the effect of increasing W_i so that W_t of the summer/autumn growth remained approximately constant at $13.9\ \text{mmol mol}^{-1}$ in the 1915–2007 period (Fig. 3b).

Correlation of $^{13}\Delta$ with environmental parameters

$^{13}\Delta$ was correlated with environmental parameters (Table 1) to identify potential causes for the substantial interannual variation of $^{13}\Delta$. PAW could only be estimated for the 1947–2007 period. Rainfall was used for the analysis of the whole period since 1857. Mean PAW in June explained 51% ($P < 0.0001$) of the variation in $^{13}\Delta$ of the spring growth in the 1947–2007 period (Fig. 4a). Mean daily rainfall from March to June explained 33% of the variation in $^{13}\Delta$ for the 1857–2007 period ($P < 0.0001$). Mean PAW from July to September explained 40% ($P < 0.0001$) of the variation in $^{13}\Delta$ of the summer/autumn growth for the 1947–2007 period (Fig. 4b), but no correlation was found with mean daily rainfall (1875–2007).

When the correlation between $^{13}\Delta$ and atmospheric CO_2 concentration was analysed separately in dry and wet years (determined by mean daily rainfall), no significant correlation in either dry or wet years was found for the 1857–2007 period for the first cut, nor for the 1875–2007 period for the second cut.

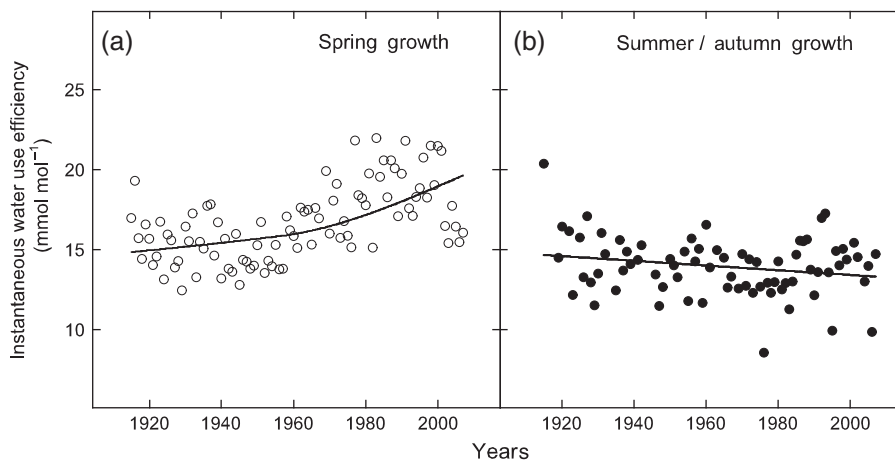


Fig. 3 Trends in instantaneous water-use efficiency (W_t) for (a) spring (○) and (b) summer/autumn growth (●) since 1915. W_t was calculated according to Eqn (4) from intrinsic water-use efficiency and the leaf-to-air difference in the mole fraction of water vapour [v , Eqn (9)]. The line in (a) gives the modelled W_t values using modelled W_i and v , (mean March–June) estimated from linear regression against year ($v = -0.0014\ \text{year} + 6.15$). W_t of the spring growth increased from 14.8 to $19.6\ \text{mmol mol}^{-1}$ (+33%). The line in (b) is the best-fit linear regression for W_t of summer/autumn growth against year ($W_t = -0.0147\ \text{year} + 42.8586$, $P = 0.07$). W_t of summer/autumn growth remained constant at about $13.9\ \text{mmol mol}^{-1}$.

Table 1 Regression statistics of the relationships between $^{13}\Delta$ and mean environmental parameters (atmospheric CO₂ concentration, air temperature, daily precipitation, vapour pressure deficit, daily plant available soil water) for the first and second cut

Parameter (unit)	Spring growth				Summer/autumn growth				R ² , P
	Period	Months	Equation	R ² , P	Period	Months	Equation	R ² , P	
CO ₂ concentration (μmol mol ⁻¹)	1857–2007	Yearly mean, seasonally corrected	$^{13}\Delta = 0.0015 [\text{CO}_2] + 20.5$	0.01, 0.3	1875–2007	Yearly mean, seasonally corrected	$^{13}\Delta = 0.0064 [\text{CO}_2] + 19.7$	0.10, <0.01	
Temperature (°C)	1878–2007	June	$^{13}\Delta = -0.1014 \text{ TEMP} + 22.4$	0.06, <0.01	1878–2007	August	$^{13}\Delta = -0.1098 \text{ TEMP} + 23.4$	0.10, <0.05	
Precipitation (mm day ⁻¹)	1857–2007	March–June	$^{13}\Delta = 0.8547 \ln(\text{RAIN}) + 20.5$	0.33, <0.0001	1875–2007	September	$^{13}\Delta = 0.2283 \ln(\text{RAIN}) + 21.6$	0.09, <0.01	
Vapour pressure deficit (kPa)	1915–2007	June	$^{13}\Delta = -2.3718 \text{ VPD} + 22.1$	0.30, <0.0001	1915–2007	July–November	$^{13}\Delta = -4.0404 \text{ VPD} + 23.2$	0.25, <0.0001	
Plant available soil water (mm)	1947–2007	June	$^{13}\Delta = 1.7156 [1 - \exp(-0.0266 \text{ PAW})] + 19.7$	0.51, <0.0001	1947–2007	July–September	$^{13}\Delta = 3.9817 (1 - \exp[-0.0779 \text{ PAW}]) + 18.2$	0.40, <0.0001	

For the atmospheric CO₂ concentration, the season-corrected yearly mean (+3.4 μmol mol⁻¹ for the spring season, -4.7 μmol mol⁻¹ for the summer/autumn season) was used. For air temperature, daily precipitation, vapour pressure deficit and plant available soil water, regressions were calculated for each month during the growing period as well as for the mean value of the growing period (March–June for spring growth, July–November for summer/autumn growth). Only the relationships, which provided the best-fit as indicated in column 'Months', are shown in the table.

Wet spring was defined as mean daily rainfall in March–June >2 mm and dry spring was defined as mean daily rainfall in March–June <1.5 mm. Wet autumn was defined as mean daily rainfall in September >3 mm and dry autumn was defined as mean daily rainfall in September <1 mm.

Discussion

The near-constancy of $^{13}\Delta$ -trends: decreasing stomatal conductance or increasing assimilation?

Carbon isotope discrimination ($^{13}\Delta$) of the unfertilized control plot of the Park Grass Experiment did not show a long-term trend for the first cut (1857–2007) but it increased slightly, although significantly, for the second cut (1875–2007). These responses meant that the c_i/c_a ratio was near-constant in the first cut and increased slightly in the second cut, provided that the relationship between $^{13}\Delta$ and the c_i/c_a ratio as predicted by Eqn (1) was accurate. It has been noted that Eqn (1) is a simplification, as it supposes that mesophyll conductance (g_m) is unlimited (Farquhar *et al.*, 1989; Seibt *et al.*, 2007). However, g_m is finite and variable (Flexas *et al.*, 2008), complicating to some extent the inference of $^{13}\Delta$ on the c_i/c_a ratio (Seibt *et al.*, 2007). Yet, g_s and g_m are generally correlated, increasing and decreasing in parallel across different plant species (Loreto *et al.*, 1992), genotypes (Lauteri *et al.*, 1997) and stress conditions (Centritto *et al.*, 2003; Flexas *et al.*, 2004). Further, in the present study the species composition, with regard to the proportion of grasses, legumes and forbs, was approximately stable over time (Silvertown *et al.*, 2006), minimizing opportunities for changes of g_m (and related effects on W_i) which might result from changes in species composition and related effects on photosynthetic traits. Therefore, we feel that the relationship between $^{13}\Delta$ and c_i/c_a ratio, as presented here, was actually quite stable.

The relative constancy of c_i/c_a ratio (and therefore $^{13}\Delta$) during rising CO₂ concentration implies that stomatal conductance has decreased and/or assimilation has increased [Eqns (1) and (3)]. Yields on plot 3 had not increased by 1992 (Jenkinson *et al.*, 1994) nor thereafter (data not shown). As photosynthesis and yield are related, and yield did not change, we should expect that stomatal conductance decreased in the face of increasing CO₂. Lack of a yield- and photosynthesis-response was probably due to nutrient-limitation of this unfertilized plot (Reich *et al.*, 2006). Nitrogen-limitation on plot 3 is due to higher N removal as compared with N input: The total sum of nitrogen removal in the form of harvested biomass for both cuts in the 1876–2001 period was estimated to be 4020 kg N ha⁻¹, as opposed

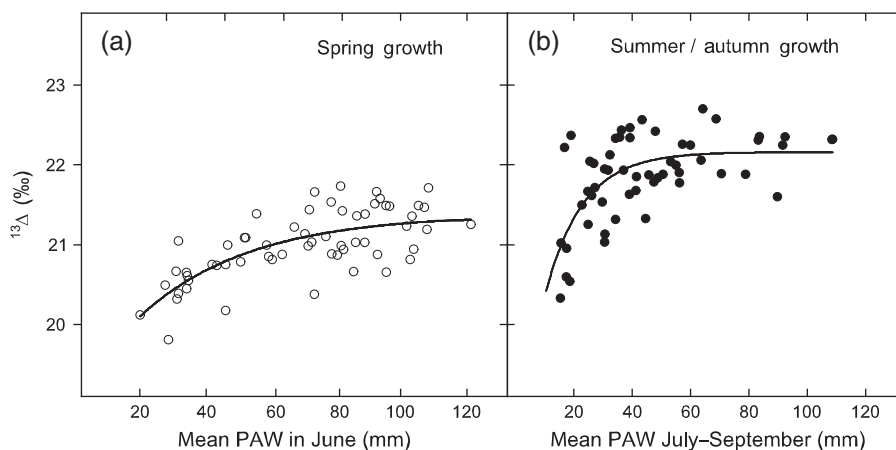


Fig. 4 ^{13}C discrimination of (a) spring and (b) summer/autumn growth as related to mean plant available soil water PAW in (a) June and (b) July–September. $^{13}\Delta$ showed a saturation response, approaching 21.4‰ for spring growth [$^{13}\Delta = 19.6671 + 1.7156(1 - \exp(-0.0266 \text{ PAW}_{\text{June}}))$, $R^2 = 0.51$] and 22.2‰ for summer/autumn growth [$^{13}\Delta = 18.1779 + 3.9817(1 - \exp(-0.0779 \text{ PAW}_{\text{July–Sept}}))$, $R^2 = 0.40$].

to $3460 \text{ kg N ha}^{-1}$ estimated atmospheric nitrogen deposition as calculated according to Goulding *et al.* (1998) for the same period. Currently, total annual N offtake through biomass is ca. 40 kg N ha^{-1} and atmospheric N input is ca. 35 kg N ha^{-1} .

Also, in one 10 years-long FACE study in a temperate climate, elevated CO_2 had no effect on herbage yield when nitrogen was added at a moderate rate, whereas addition of nitrogen fertilizer at a high rate sustained a strong enhancement of aboveground biomass production of *L. perenne* L. swards (Schneider *et al.*, 2004). Such responses are the rule, although they are not always observed (Reich *et al.*, 2006). A stronger acclimation response of photosynthesis under nitrogen limitation was also observed repeatedly (Ainsworth & Rogers, 2007). Together, these features all support the view that the long-term constancy of $^{13}\Delta$, and c_i/c_a ratio, in the present nutrient-limited grassland was mainly achieved via a decrease of stomatal conductance. Moreover, they demonstrate that this response was sustained over a period of more than one century.

Intrinsic water-use efficiency (W_i) vs. instantaneous water-use efficiency (W_t)

The $^{13}\Delta$ and c_a data showed that W_i increased by 33% in the first cut and by 18% in the second cut, indicating that W_i of this temperate grassland system has adjusted physiologically to the rising CO_2 concentration of the last century. Interestingly, the response was similar to that observed in long-term isotope studies on trees (Bert *et al.*, 1997; Duquesnay *et al.*, 1998; Feng, 1999; Saurer *et al.*, 2004) suggesting that there may be no fundamental difference between (temperate and northern latitude)

forests and temperate grasslands in the response of intrinsic water-use efficiency to increasing CO_2 .

The increased intrinsic water-use efficiency of the first cut was probably translated into the same increase of W_t , because spring-time VPD (March–June) did not change. In total, both increased by approximately 30% (1915–2007). If we assume that assimilation has remained unchanged (as above) leaf-level transpiration must have decreased accordingly. Furthermore, if the allometry of aboveground biomass and leaf area did not change, then system-scale transpiration must have decreased in the same way, increasing the likelihood of groundwater recharge and runoff losses of water in spring time. Conversely, the increase of W_i during summer/autumn growth (+18%) was probably completely offset by the increased VPD. Thus W_t remained unchanged. Again, if we argue in the same way as above, then transpiration probably did *not* decrease in the summer/autumn period. Thus, system-scale transpiration would have decreased in spring but not in summer/autumn, changing the seasonal cycle of transpiration.

However, these conclusions are based on the assumption that leaf temperature followed air temperature. Decreasing stomatal conductance and therefore decreasing transpiration could lead to increasing leaf temperature, thus increasing the VPD between leaf intercellular spaces and the atmosphere. We estimated for the spring growth that leaf temperature must have increased by 1.3°C above ambient to offset the increase in W_t .

Absolute values of W_t were relatively high, as the mean leaf-to-air difference in the mole fraction of water vapour at the study site was comparatively low (mean

value for the 1915–2007 period from March to June: $3.3 \pm 0.4 \text{ mmol mol}^{-1}$ and for July–November: $3.5 \pm 0.7 \text{ mmol mol}^{-1}$.

Correlation of $^{13}\Delta$ with environmental parameters

Interannual and intraannual variations of $^{13}\Delta$ were most strongly correlated with PAW. Such a close relationship between PAW and community-level $^{13}\Delta$ has been observed before (Schnyder *et al.*, 2006), but has not been analysed in long series of climatic observations in grassland. Correlations with other weather factors, such as precipitation and VPD, were also evident and agreed with those observed by others (Korol *et al.*, 1999; Hemming *et al.*, 2005; Schulze *et al.*, 2006). However, relationships of $^{13}\Delta$ with precipitation and VPD were weaker and less consistent than those with PAW. So, it does seem that the closer relationship between PAW and $^{13}\Delta$ was due to the more integrative nature of this parameter, which accounted for the effects of evaporative demand and precipitation on canopy hydraulics. Certainly, the present observation of a strong relationship between PAW and $^{13}\Delta$ calls for a careful interpretation of CO₂ effects on $^{13}\Delta$, as these could result (indirectly) from the antitranspirant effect of CO₂ on PAW.

Conclusion

This work presents a long-term series of community-level carbon isotope composition of a temperate nutrient-limited grassland. We showed that community-level $^{13}\Delta$ was constant or increased slightly during the increase in the concentration of atmospheric CO₂ over the last 150 years. This suggested that W_i increased substantially during spring and summer/autumn growth, consistent with observations on trees. However, the effect of CO₂ on W_i was strongly modified by VPD. Spring-time VPD did not change since 1915, so that W_i and W_t increased in the same way (+ 30% in both cases). In contrast, VPD during summer/autumn growth increased after 1915, completely negating the effect of increased W_i on W_t . However, increases in leaf temperature might have offset some of the antitranspirant effect of elevated CO₂. The dataset revealed significant intra- and inter-annual variation in $^{13}\Delta$ which was closely related to variation in PAW.

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