

Nutrient supply enhanced the increase in intrinsic water-use efficiency of a temperate seminatural grassland in the last century

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Abstract

Under the increase in atmospheric CO₂ during the last century, variable increases in the intrinsic water-use efficiency (W_i), i.e., the ratio between carbon assimilation rate (A) and stomatal conductance (g_s), of C₃ vegetation have been observed. Here, we ask if long-term nutrient status and especially nitrogen supply have an effect on the CO₂ response of W_i in a temperate seminatural C₃ grassland. This analysis draws on the long-term trends (1915–2009) in W_i , derived from carbon isotope analysis, of archived hay and herbage from the Park Grass Experiment at Rothamsted (South-East England). Plant samples came from five fertilizer treatments, each with different annual nitrogen (N; 0, 48 or 96 kg ha⁻¹), phosphorus (P; 0 or 35 kg ha⁻¹) and potassium (K; 0 or 225 kg ha⁻¹) applications, with lime as required to maintain soil pH near 7. Carbon isotope discrimination ($^{13}\Delta$) increased significantly ($P < 0.001$) on the Control (0.9‰ per 100 ppm CO₂ increase). This trend differed significantly ($P < 0.01$) from those observed on the fertilized treatments (PK only: 0.4‰ per 100 ppm CO₂ increase, $P < 0.001$; Low N only, Low N+PK, High N+PK: no significant increase). The $^{13}\Delta$ trends on fertilized treatments did not differ significantly from each other. However, N status, assessed as N fertilizer supply plus an estimate of biologically fixed N, was negatively related ($r^2 = 0.88$; $P < 0.02$) to the trend for $^{13}\Delta$ against CO₂. Other indices of N status exhibited similar relationships. Accordingly, the increase in W_i at High N+PK was twice that of the Control (+28% resp. +13% relative to 1915). In addition, the CO₂ responsiveness of $^{13}\Delta$ was related to the grass content of the plant community. This may have been due to the greater CO₂ responsiveness of g_s in grasses relative to forbs. Thus, the greater CO₂ response of grass-rich fertilized swards may be related to effects of nutrient supply on botanical composition.

Keywords: atmospheric CO₂, C₃ grassland, carbon isotope discrimination ($^{13}\Delta$), climate change, functional group, hay and herbage, nitrogen, Park Grass Experiment, water-use efficiency

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Introduction

Global biogeochemical carbon and water cycles are affected by rising atmospheric CO₂ and its impacts on terrestrial plants' gas exchange (Gedney *et al.*, 2006; Betts *et al.*, 2007). Direct and compelling evidence for changes in carbon uptake relative to potential water loss by plants under the recent increase in atmospheric CO₂ comes from time-series analysis of the intrinsic water-use efficiency (W_i) of forest trees and C₃ grassland vegetation during the 20th century (Bert *et al.*, 1997; Duquesnay *et al.*, 1998; Feng, 1999; Arneeth *et al.*, 2002; Saurer *et al.*, 2004; Barbosa *et al.*, 2010; Köhler *et al.*, 2010; Andreu-Hayles *et al.*, 2011; Peñuelas *et al.*, 2011). W_i is a physiological efficiency reflecting the leaf-level relationship between plant CO₂ uptake and potential transpiration i.e. transpiration at a standard vapour

pressure deficit. Thus, W_i represents the ratio of carbon assimilation rate (A) to stomatal conductance (g_s), which determines the CO₂ concentration gradient between the atmosphere (c_a) and the internal leaf gas space (c_i) (Eqn 1). Although the direct measurement of c_i is not possible, its value in C₃ plants can be estimated from the known relationship between c_i/c_a and carbon isotope discrimination ($^{13}\Delta$) in C₃ photosynthesis, using the linear Farquhar model (see Eqn 2 and Farquhar *et al.*, 1989); c_a is obtained from measurements of CO₂ in free air (Keeling *et al.*, 2009) and in gas bubbles from ice cores for the time before CO₂ measurements were made (Friedli *et al.*, 1986; Francey *et al.*, 1999). Thus, with $A = g_{\text{CO}_2} (c_a - c_i)$ (according to Fick's law) and with $g_{\text{H}_2\text{O}} = 1.6 g_{\text{CO}_2}$ we can relate $^{13}\Delta$ to W_i via Eqns 1 and 2;

$$W_i = \frac{A}{g_{\text{H}_2\text{O}}} = \frac{c_a - c_i}{1.6} = \frac{c_a \cdot (1 - \frac{c_i}{c_a})}{1.6}, \text{ with } \quad (1)$$

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$$\frac{c_i}{c_a} = \frac{{}^{13}\Delta - a}{b - a} \quad (2)$$

where 1.6 is the ratio between the diffusivities of water vapour and CO₂ in air (Eqn 1), *a* denotes the fractionation of ¹³CO₂ (relative to ¹²CO₂) that occurs during diffusion in air (4.4‰) and *b* is the net fractionation due to carboxylations (27‰) (Eqn 2). ¹³Δ is obtained from the carbon isotope composition of plant material (δ¹³C_p) and that of the atmosphere (δ¹³C_a; Friedli *et al.*, 1986; Francey *et al.*, 1999; White & Vaughn, 2011) as

$${}^{13}\Delta = (\delta^{13}\text{C}_a - \delta^{13}\text{C}_p)/(1 + \delta^{13}\text{C}_p). \quad (3)$$

An increasing ¹³Δ enhances the disparity between δ¹³C_a and δ¹³C_p. Using ¹³Δ (estimated by Eqn 3), carbon isotope analysis of tree-rings (e.g., McCarroll & Loader, 2004; Peñuelas *et al.*, 2011) or archived grassland herbage samples (Köhler *et al.*, 2010) have enabled retrospective studies of *W*_i as determined by Eqns 1 and 2.

Studies of ¹³Δ in forest trees and grassland often found increases in *W*_i with rising CO₂ (Bert *et al.*, 1997; Duquesnay *et al.*, 1998; Feng, 1999; Arneth *et al.*, 2002; Saurer *et al.*, 2004; Barbosa *et al.*, 2010; Köhler *et al.*, 2010; Andreu-Hayles *et al.*, 2011; Peñuelas *et al.*, 2011) across a wide range of locations (e.g., the Iberian Peninsula, Western/Northern Europe, Siberia or North America), tree species (e.g., from the *Pinus*, *Quercus*, *Picea* or *Fagus* genera) and grassland types; few studies found no change in *W*_i (Marshall & Monserud, 1996; Betson *et al.*, 2007). Others observed that the increase in *W*_i has levelled off in recent decades (Waterhouse *et al.*, 2004; Gagen *et al.*, 2011). In addition, others found that the long-term change in *W*_i within the same tree species or genus differed between study sites (Peñuelas *et al.*, 2008; *Fagus sylvatica*; Andreu-Hayles *et al.*, 2011; *Pinus sp.*). The great variability in the response of *W*_i to rising atmospheric CO₂ suggests interactions with other environmental factors. Little is known about the actual identity and quantitative effects of such factors. These could include different hydrological conditions (e.g., plant available soil water), differences in climate (e.g., arid vs. humid) and nutrient status (high vs. low nutrient availability). Such factors could affect *W*_i via changes of *A* or *g*_s or both and may thus enhance or counteract the CO₂ response of *W*_i. A particular difficulty in the study of such interactions is the scarcity of long-term experiments that can be used to examine the influence of these environmental factors.

The present work is concerned with the long-term effects of nutrient availability, especially N supply, on *W*_i. Shorter term experiments and FACE studies have demonstrated interactions between the CO₂ responses of photosynthesis and stomatal conductance with other

environmental factors, including N (Stitt & Krapp, 1999; Ainsworth & Rogers, 2007). To our knowledge only Betson *et al.* (2007) have investigated the role of nutrient supply on the long-term CO₂ response of *W*_i. They analysed ¹³Δ in needles of *Pinus sylvestris* from a 32 years long N loading experiment and found no significant increase in *W*_i on the control and N fertilizer treatments. Apart from this, very little is known about the long-term interaction between rising atmospheric CO₂ and nutrient supply and its effects on *W*_i at the ecosystem level.

Here, we analyse long-term records of ¹³Δ in archived hay and herbage samples from five of the fertilizer treatments on the Park Grass Experiment (PGE) (Silvertown *et al.*, 2006) at Rothamsted, England. This experiment comprises various treatments, including different combinations and rates of N, P and K fertilizers applied annually to the same experimental plots since 1856. Hay or herbage samples from the plots have been archived each year since the establishment of the experiment. Consequently, they can be used to examine the interactive effects of climate change and nutrient supply on grassland community-level ¹³Δ and *W*_i at a single site, avoiding the complications associated with multi-site comparisons.

In this study we examine whether nutrient supply (i.e., different amounts and combinations of N, P and K fertilizers), and in particular the supply of N, affected the long-term CO₂ response of grassland between 1915 and 2009, with respect to changes in ¹³Δ, *c*_i/*c*_a and *W*_i.

Materials and methods

Experimental site and fertilizer treatments

The PGE began 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 was divided into 20 experimental plots, which received different fertilizer and manure treatments. Later, tests of lime were also introduced on the plots. The experiment is located on a moderately well-drained silty clay loam overlying clay-with-flints. The soil pH was slightly acidic when the experiment began (5.4–5.6) and the nutrient status was poor (Silvertown *et al.*, 2006). The original vegetation of the PGE has been classified by Dodd *et al.* (1998) as dicotyledon-rich *Cynosurus cristatus-Centaurea nigra* grassland, which is one of the mesotrophic grassland communities in the British National Vegetation Classification system. Since the start of the experiment the herbage has been cut around mid June (Cut 1) and made into hay; samples were taken from the material dried *in situ* until 1960. In subsequent years strips were cut with a forage harvester and fresh herbage samples were taken for drying and archiving. The herbage remaining on the plot was made into hay, as before. Originally the subsequent regrowth was grazed

by sheep penned on individual plots, but after 1875, grazing ceased and a second harvest cut (Cut 2) was removed green. Dried samples from all plots and both cuts have been stored in the Rothamsted Sample Archive since the beginning of the experiment (Silvertown *et al.*, 2006).

Treatments with contrasting nutrient inputs, where soil pH was relatively stable over time and was not negatively affecting plant growth, were selected for this study. The treatments chosen include the unfertilized control together with low ($48 \text{ kg ha}^{-1} \text{ a}^{-1}$) and high ($96 \text{ kg ha}^{-1} \text{ a}^{-1}$) N fertilizer with or without additional P ($35 \text{ kg ha}^{-1} \text{ a}^{-1}$ as triple superphosphate) and K ($225 \text{ kg ha}^{-1} \text{ a}^{-1}$ as potassium sulphate), hereafter referred to as *Control*, *PK*, *Low N*, *Low N+PK* and *High N+PK* treatments respectively (see details in Table 1). Most treatments have not been replicated nor randomized. However, the meadow was reasonably uniform before the experiment began (Crawley *et al.*, 2005) and the size of the plots ($450\text{--}1910 \text{ m}^2$) compensates to some extent for the lack of replication. Only for the *Control* and the *PK* treatment were replicate plots available. Thus, hay and herbage samples from seven plots (encompassing five treatments) were studied. Fertilizer N is applied as sodium nitrate in spring; other minerals are applied in winter. For this study we analysed only the first cut (spring/summer growth) as it responds most to fertilizer application. In addition to the fertilizer treatments a test of lime was introduced on Park Grass from 1903; the main treatment plots were split into unlimed (*U*) and limed (*L*) areas, and the latter received chalk (4 t ha^{-1}) every fourth year. Four of the seven selected plots [plot 2 and 3 (*Control*), 7 (*PK*) and

16 (*Low N+PK*)] received chalk in this way from 1903 and the three remaining plots [15 (*PK*), 17 (*Low N*) and 14 (*High N+PK*)] were limed from 1920 onward. In 1965 the treatments were further divided into 'a', 'b', 'c' and 'd' subplots, which received different amounts of chalk to maintain the soil (0–23 cm) at a pH (in water) of about 7, 6 and 5 on the *a*, *b* and *c* subplots; the *d* subplots received no chalk so that the soil pH reflects inputs from the different fertilizers and atmospheric deposition only. The soil pH of the limed (*L* and *a*) subplots used in this study varied between 6.0 and 7.5.

Botanical composition of the swards differed *between* treatments, but the functional groups *within* treatments were relatively constant: According to Silvertown *et al.* (2006), by 1910 the botanical composition had reached a dynamic equilibrium at the functional group level; subsequent interannual changes were due to yearly differences in climate. The *Control* treatment showed a high contribution of forbs (40%), whereas the *PK* treatment contained a substantial amount of legumes (26%). The *Low N*, *Low N+PK* and *High N+PK* treatments were dominated by grasses (62%, 67% and 83%); for a summary and for dominant species see Table 1.

Sample preparation, $\delta^{13}\text{C}$ and elemental analysis

Representative subsamples of plant material, each of 2–3 g, were taken from archived hay or herbage samples from the selected treatments (Table 1). 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 $0.7 \pm 0.05 \text{ mg}$

Table 1 Details of fertilizer application, botanical composition and dry matter production on selected treatments of the Park Grass Experiment

Treatment* [number of analysed samples]	Annual nutrient applications; N, P and K (kg ha^{-1})	Percent contribution of functional groups ($\pm\text{SD}$) to harvested herbage mass† [number of years where data were available]	Dominant species‡ in 1991–2000 [total number of species]	Cut 1 mean dry matter production§ for 1960–2009 ($\text{t ha}^{-1} \pm \text{SD}$)
<i>Control</i> [186]	Nil	G: 49 ± 12 F: 40 ± 11 L: 10 ± 5 [44]	<i>Agrostis capillaris</i> , <i>Festuca rubra</i> , <i>Leontodon</i> <i>hispidus</i> [44]	2.1 ± 0.6
<i>PK</i> [185]	P: 35 K: 225	G: 54 ± 15 F: 20 ± 11 L: 26 ± 14 [47]	<i>Arrhenaterium elatius</i> , <i>F. rubra</i> , <i>Lathyrus pratensis</i> , <i>Trifolium pratense</i> [30]	4.7 ± 0.8
<i>Low N</i> [92]	N: 48	G: 62 ± 18 F: 36 ± 18 L: 2 ± 2 [21]	<i>A. capillaris</i> , <i>F. rubra</i> , <i>L. hispidus</i> , <i>Plantago</i> <i>lanceolata</i> [36]	2.7 ± 0.6
<i>Low N+PK</i> [94]	N: 48 P: 35 K: 225	G: 67 ± 15 F: 21 ± 11 L: 12 ± 10 [15]	<i>A. elatius</i> , <i>Alopecurus</i> <i>pratensis</i> , <i>F. rubra</i> [24]	5.0 ± 0.6
<i>High N+PK</i> [89]	N: 96 P: 35 K: 225	G: 83 ± 11 F: 15 ± 11 L: 2 ± 3 [16]	<i>A. elatius</i> , <i>A. pratensis</i> [27]	5.1 ± 0.9

*Chalk was applied as required to maintain soil pH between 6.0 and 7.0.

†Mean botanical composition at the functional group level [grasses (G), nonlegume forbs (F) and legumes (L)] is calculated from botanical separation data available for individual years in the 1915–1976 period and for each year in the 1991–2000 period from eRA (2011).

‡Species comprising more than 10% of herbage (Cut 1) in the 1991–2000 botanical surveys (eRA, 2011).

§From forage harvester yield.

were weighed into tin cups (IVA Analysentechnik e.K., Meerbusch, Germany) and combusted in an elemental analyser (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 CO_2 gas, which was previously calibrated against a secondary isotope standard (IAEA-CH6 for ^{13}C , accuracy of calibration $\pm 0.06\%$ SD). After every tenth 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 0.11‰ (SD of 190 SILS replications). Carbon and N elemental concentrations (%C and %N) and isotope composition ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) were measured in the same sequence, but the %C and $\delta^{15}\text{N}$ data were not needed for this study.

We also tested if the change in the harvesting method in 1960 had systematically affected $\delta^{13}\text{C}$ values. The *Control*, the *Low N+PK* and the *High N+PK* treatments were harvested with both methods in a comparative sampling in 1992, 1993 and 1994. Differences between $\delta^{13}\text{C}$ in forage and hay samples were small and inconsistent. Thus, we assumed that $\delta^{13}\text{C}_p$ values prior to 1960 were not systematically biased by sampling as hay and we used the original data for our analyses.

Measures of N status

The trends of $^{13}\Delta$ resp. c_i/c_a of botanically diverse grassland swards were compared with several measures of N status: N input from fertilizer and biologically fixed N, the N nutrition index (NNI) and the Ellenberg N indicator value.

N input was determined as fertilizer N supply on the *Low N*, *Low N+PK* and *High N+PK* treatment. Additional input by biological N fixation was taken into account on the legume-rich *PK* and *Low N+PK* treatments. Data on the percentage of legumes in the swards were available for individual years between 1915 and 1976 and for each year from 1991 to 2000 (eRA, 2011). The average annual N input by biological N fixation was calculated using the Høgh-Jensen model (Høgh-Jensen *et al.*, 2004) with the parameterization for cut 1–2 years old grass-red clover systems.

The NNI was calculated according to Lemaire & Gastal (1997) as the ratio of actual (N_{act}) to critical (N_{crit}) N concentration in the sward, where $N_{\text{crit}} = 4.8(W)^{-0.32}$, and W is above-ground biomass (t ha^{-1}). N_{crit} is the minimum N concentration which allows maximum growth rate. NNI was calculated for biomass harvests obtained after 1960, as prior samplings underestimated W to some degree (Jenkinson *et al.*, 1994). Pre-1960 samplings were made from the material dried *in situ*, which is affected by disintegration losses in the hay making process (e.g., shattered leaves). Data for harvested above-ground biomass (t ha^{-1}) were available from the electronic Rothamsted Archive (eRA, 2011) and actual N concentration (%) in herbage came from measurements of %N in the plant samples.

Species indicator values can be used to draw conclusions about effects of environmental factors (e.g., light, soil moisture, soil pH and soil N) on the plant species composition of communities. Commonly used indicator values for vascular plants are the so-called Ellenberg indicator values (for a review see Diekmann, 2003). We calculated the average Ellenberg N indicator values of communities (weighted by species biomass) for each treatment. Whole-number gradation from 1 to 9 indicates increasing N availability: species with an N value of 9 indicate excessively N-rich sites, whereas 1 indicates extremely N-poor sites, with 5 indicating moderately N-rich sites. The recalibrated Ellenberg values for British plants were used (Hill *et al.*, 1999). Data on the percentage contribution of separate species to the total mass of the harvested herbage are available for individual years during the 1915–1976 period and for each year in the 1991–2000 period (eRA, 2011).

Climatic data and calculation of plant available soil water for the standard grass reference crop (PAW_{ref})

Climatic data and trends were analysed in Köhler *et al.* (2010): mean annual rainfall was 735 mm (± 120 mm SD) between 1857 and 2007 (no significant long-term trend was observed); mean annual temperature has increased significantly since the beginning of the recording in 1878 (after 1990 the increase was more pronounced); mean annual temperature in the 1878–1989 period was 9.1 °C (in the 1990–2007 period it was 10.2 °C) and no trend was observed in the vapour pressure deficit (VPD) during spring growth ($P = 0.4$). Plant available soil water (PAW) has been shown to explain inter- and intra-annual variation in $^{13}\Delta$ better than single climatic factors like VPD or precipitation (Schnyder *et al.*, 2006; Köhler *et al.*, 2010). PAW was calculated as $\text{PAW}_i = \text{PAW}_{i-1} + P_i - \text{AET}_i$, where PAW_{i-1} is the modelled plant available water 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 $\text{PAW}_i/\text{PAW}_{\text{capacity}} > 0.3$. Otherwise AET_i was calculated as $\text{AET} = \frac{\text{PET}}{0.3} \cdot \frac{\text{PAW}_i}{\text{PAW}_{\text{capacity}}}$ (Schnyder *et al.*, 2006). PET was estimated with the FAO Penman–Monteith equation for a standard grass reference crop (Allen *et al.*, 1998). The estimated PAW provides a standard measure for the overall evaporative conditions and water availability in individual years, but does not account for eventual treatment effects on PAW. The parameter is therefore termed PAW_{ref} (plant available soil water for the standard grass reference crop) in the subsequent text. Daily PAW_{ref} values were averaged over different periods of time during the growing season to find the best relationship with $^{13}\Delta$. The maximum plant available soil water ($\text{PAW}_{\text{capacity}}$) of the soil has not been measured directly, but was inferred to be 135 mm (for the top 70 cm of the soil) from measurements on similar soils at Rothamsted (Avery & Catt, 1995). Modelling was started on the 15th of February in 1915 when PAW_i was set to 135 mm, as the soil was most likely saturated with water at that time of the year. For the calculation of PET, windspeed was estimated from windforce (Beaufort scale, 1915–1947) and windrun (km per day, 1948–1959), when windspeed measurements (m s^{-1} , 1960–2009) were not available. All other weather parameters neces-

sary for the calculation were available for the 1915–2009 period (eRA, 2011).

Statistical data analysis

All statistical analysis was done with *R* (R Development Core Team, 2012). Long-term trends and interannual variation in $^{13}\Delta$ were analysed using linear regression. We used a multiple linear regression model with atmospheric CO_2 concentration [CO_2] and the natural logarithm of PAW_{ref} as explanatory variables to assess long-term trends in $^{13}\Delta$. We used a logarithmic transformation of PAW_{ref} , as the increase in $^{13}\Delta$ with increasing PAW followed closely a logarithmic relationship in the observed range (see e.g., Schnyder *et al.*, 2006; Köhler *et al.*, 2010). Furthermore, treatment (*Control*, *Low N*, *PK*, *Low N+PK*, *High N+PK*) was included as a factor. The model

$$^{13}\Delta = \beta_0 + \beta_1 \ln(\text{PAW}_{\text{ref}}) + \beta_2 \text{Treatment}[\text{CO}_2] \quad (4)$$

was fitted to the data, where β_0 , β_1 and β_2 are fitting parameters to be estimated. The *multcomp* package (Hothorn *et al.*, 2008) in *R* was used for multiple comparisons of model parameters.

Results

Long-term trends in $\delta^{13}\text{C}$

The $\delta^{13}\text{C}$ values of the hay and herbage samples ($\delta^{13}\text{C}_p$) are presented for the 1915–2009 period in Fig. 1, together with the trend in $\delta^{13}\text{C}$ of atmospheric CO_2 ($\delta^{13}\text{C}_a$; for the model see Köhler *et al.*, 2010). The decrease in $\delta^{13}\text{C}_p$ largely reflects the decreasing $\delta^{13}\text{C}_a$ (Suess effect), with all treatments following a similar pattern. The $\delta^{13}\text{C}_p$ values were in the range of -26.0‰ to -29.8‰ on the *Control* treatment. Similar ranges were observed on the fertilized treatments (*Low N*: -26.2‰ to -29.1‰ ; *PK*: -25.9‰ to -29.6‰ ; *Low N+PK*: -26.3‰ to -29.4‰ ; *High N+PK*: -26.1‰ to -29.4‰).

$^{13}\Delta$ values and the relationship with PAW_{ref}

$^{13}\Delta$ values were estimated using Eqn 3. Differences between minimum and maximum $^{13}\Delta$ values within each treatment varied between 1.8‰ and 2.7‰ (*Control*: 2.3‰ ; *Low N*: 1.8‰ ; *PK*: 2.5‰ ; *Low N+PK*: 1.8‰ ; *High N+PK*: 2.7‰). Mean $^{13}\Delta$ values \pm SD of the treatments were $20.8 \pm 0.5\text{‰}$ (*Control*), $20.7 \pm 0.5\text{‰}$ (*Low N*), $20.6 \pm 0.5\text{‰}$ (*PK*), $20.7 \pm 0.4\text{‰}$ (*Low N+PK*) and $20.8 \pm 0.5\text{‰}$ (*High N+PK*).

The interannual variability was related to PAW_{ref} . The relationship between $^{13}\Delta$ and water supply was examined by averaging PAW_{ref} over different monthly periods and comparing the results. The closest relationship between $^{13}\Delta$ and PAW_{ref} was found when average June PAW_{ref} was used. This explained between 39%

(*Low N*) and 52% (*PK*) of the variation (*Control*: 41%, *High N+PK*: 44%, *Low N+PK*: 48%) on the individual treatments. The slopes and intercepts of the different treatments did not differ significantly from each other. Therefore, the data were merged for an analysis of the pooled data. The overall relationship between $^{13}\Delta$ and PAW_{ref} for the pooled data [$^{13}\Delta = 18.37 + 0.58 \ln(\text{PAW}_{\text{ref}})$], explained 44% of the variation in the entire data set. PAW_{ref} did not show any long-term change with increasing atmospheric CO_2 ($P = 0.5$). Mean PAW_{ref} was 63.6 mm.

Long-term trends in $^{13}\Delta$, c_i/c_a and W_i

The analysis of long-term trends was done with the multiple linear regression model (Eqn 4). The model explained 53% of the total variation in $^{13}\Delta$. The model

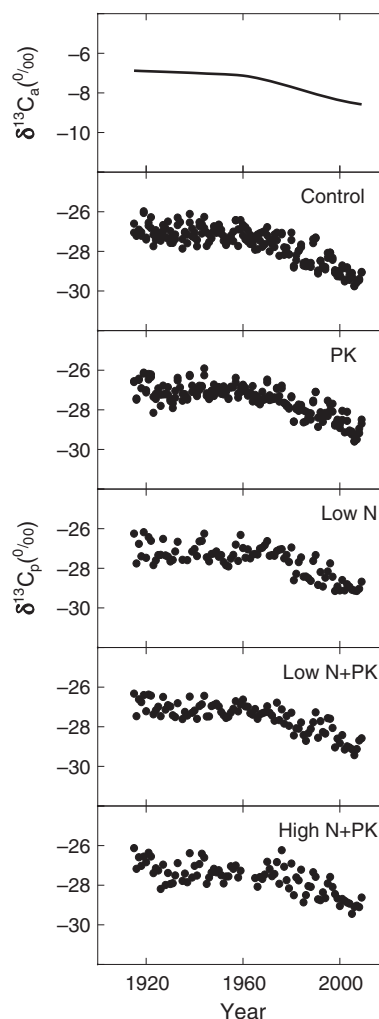


Fig. 1 Long-term trends in $\delta^{13}\text{C}$ of atmospheric CO_2 ($\delta^{13}\text{C}_a$) and harvested biomass ($\delta^{13}\text{C}_p$) from the first hay/herbage cut taken from selected fertilizer treatments on the Park Grass Experiment (see Table 1).

results for $^{13}\Delta$ ($^{13}\Delta_{\text{mod}}$) are shown separately for each treatment in Fig. 2a. Significant increases were observed on the *Control* and the *PK* treatments. The changes in $^{13}\Delta$ per 100 ppm CO_2 increase were (from highest to lowest): 0.9‰ for the *Control* ($P < 0.001$), 0.4‰ for the *PK* ($P < 0.001$), 0.3‰ for the *Low N* ($P = 0.2$) and the *Low N+PK* ($P = 0.1$) and no change for the *High N+PK* ($P = 1$) treatment. The trend lines in Fig. 2a illustrate the estimated changes in $^{13}\Delta$ with increasing atmospheric CO_2 for each treatment.

The response of $^{13}\Delta$ resp. c_i/c_a can be compared with three theoretical scenarios (Saurer *et al.*, 2004): (i) constant c_i , (ii) constant c_i/c_a or (iii) constant $c_a - c_i$. For scenarios (i) and (ii) A must increase and/or g_s must decrease, leading to increasing W_i . Scenario (i) causes a stronger increase in W_i and requires a stronger reaction of A and/or g_s than scenario (ii) [termed 'active response' by McCarroll *et al.* (2009)]. In scenario (iii), the increase in c_a is paralleled by the same increase in c_i ; thus no active response of A and/or g_s is required, ['passive response', McCarroll *et al.* (2009)] leading to unchanged W_i . When $^{13}\Delta$ was interpreted according to Eqn 2, the responses of c_i/c_a ranged between the 'constant c_i/c_a ' and 'constant $c_a - c_i$ ' scenarios (grey-shaded area in Fig. 2a). We tested if pairwise differences between the slopes of single treatments were significantly different from zero. Significant differences were only found for the *Control* treatment in comparison with the other treatments (adjusted P -values for multiple comparisons < 0.01).

W_i ranged from 51.8 (*High N+PK*) to 53.8 $\mu\text{mol mol}^{-1}$ (*PK*) in 1915 and from 60.9 (*Control*) to 66.4 $\mu\text{mol mol}^{-1}$ (*High N+PK*) in 2009. The increases on all treatments lay between 13% (*Control*) and 28% (*High N+PK*) (Fig. 2b). This meant that the possible relative maximum increases in A lay between +13% (*Control*) and +28% (*High N+PK*) and the possible relative maximum decreases in g_s lay between -22% (*High N+PK*) and -12% (*Control*).

N measures and changes in $^{13}\Delta$

The estimated annual rate of biological N fixation (\pm SE) was $50 \pm 5 \text{ kg ha}^{-1}$ on the *PK* treatment and $31 \pm 8 \text{ kg ha}^{-1}$ on the *Low N+PK* treatment. Mean N concentrations \pm SD in plant material from the studied treatments were $1.8 \pm 0.2\%$ (*Control*), $1.9 \pm 0.3\%$ (*Low N*), $1.8 \pm 0.3\%$ (*PK*), $1.6 \pm 0.2\%$ (*Low N+PK*) and $1.7 \pm 0.3\%$ (*High N+PK*). The NNI ranged between 0.47 in the *Control* and ~ 0.61 in the *PK* and *High N+PK* treatments. Intermediate NNIs were observed in the other treatments (0.55 at *Low N* and 0.56 at *Low N+PK*). In the treatments with highest NNI (*PK* and *High N+PK*), NNI decreased slightly with time and CO_2 (-0.03 resp.

-0.02 per 10 ppm CO_2 increase on the *PK* ($P < 0.001$) resp. *High N+PK* ($P < 0.05$) treatment). No such effect was noted in the other treatments. The average Ellenberg N indicator values of the treatments were: 4.0 (*Control*), 4.4 (*Low N*), 5.4 (*PK*), 5.8 (*Low N+PK*) and 6.5 (*High N+PK*), SD was < 0.3 on all treatments. The Ellenberg N indicator value did not change with time (or CO_2), except for the *Low N* treatment where a slight decrease of 0.9 was observed over the last 100 years.

Ranking the $^{13}\Delta$ responses gave the order (from biggest to smallest slope): *Control* $>$ *PK* $>$ *Low N*; *Low N+PK* $>$ *High N+PK* (see Fig. 2a). These $^{13}\Delta$ responses (slopes) were related to the different measures of N status (Fig. 3). For all three measures a similar pattern was apparent: the $^{13}\Delta$ response to CO_2 decreased with increasing N supply resp. status. This relationship was significant ($r^2 = 0.88$; $P < 0.02$) for the effect of fertilizer N input (including biologically fixed N). For the NNI and Ellenberg N indicator value significance of the relationship was only reached at the 10% significance level (NNI: $r^2 = 0.72$, $P = 0.07$; Ellenberg N indicator value: $r^2 = 0.65$, $P = 0.10$).

Discussion

This work demonstrated a significant interaction between N supply (including N fixation by legumes) and the long-term $^{13}\Delta$ response of the limed grassland in the PGE under increasing atmospheric CO_2 . Accordingly, the long-term increase in W_i on the *High N+PK* treatment ($+14.5 \mu\text{mol mol}^{-1}$) was greater than on the unfertilized *Control* treatment ($+7.2 \mu\text{mol mol}^{-1}$). The CO_2 response of $^{13}\Delta$ was also related with the NNI (an ecophysiological based indicator of canopy N status; Lemaire & Gastal, 1997), and with the Ellenberg N value (an ecological indicator value derived from observations of the occurrence of plant species along gradients of soil N availability or soil fertility; Ellenberg *et al.*, 1992; Diekmann, 2003; Hill *et al.*, 1999).

The significant effect of N supply on the $^{13}\Delta$ response of grassland to increasing atmospheric CO_2 reported here differs from that for *P. sylvestris*, as reported by Betson *et al.* (2007), the only other investigation of $\text{CO}_2 \times \text{N}$ interaction during recent climate change. In their 32 years long experiment Betson *et al.* (2007) found no effect of N supply on the CO_2 response of $^{13}\Delta$ (and W_i). Actually, W_i of *P. sylvestris* remained constant in their study, whereas we observed increasing W_i in all treatments. Taken together, the two studies indicate that the effect of N fertilizer on the CO_2 response of $^{13}\Delta$ is variable, and is possibly modified by further factors. Such factors could include interactions with other nutrients or environmental variables including soil chemistry (such as pH) and physical conditions, climatic

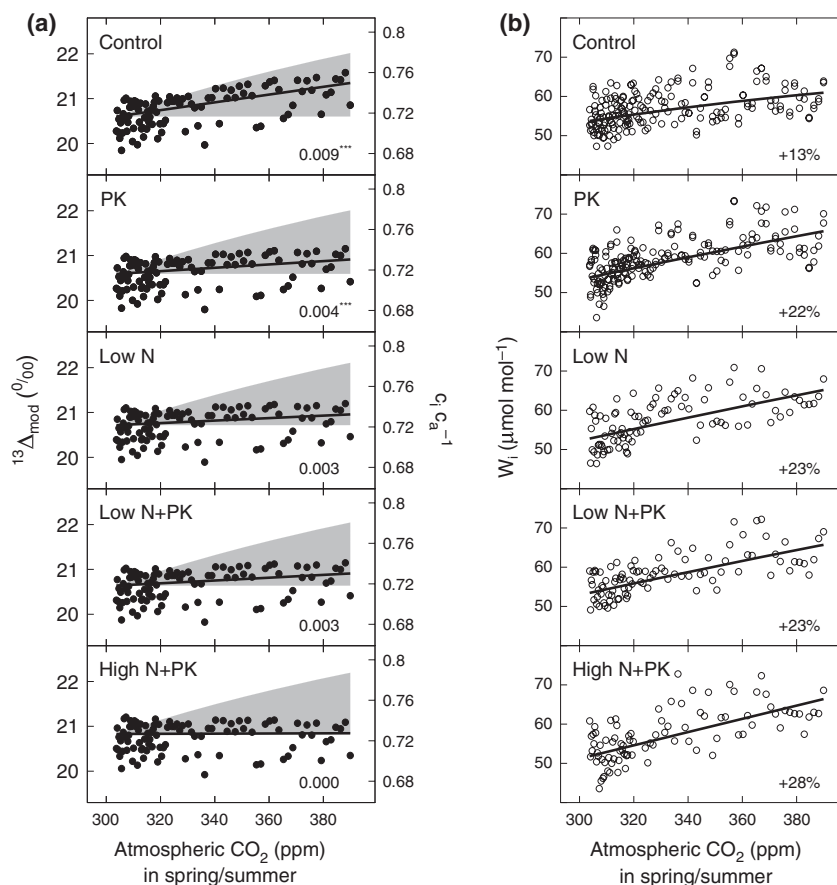


Fig. 2 (a) Modelled long-term trends in $^{13}\Delta$ ($^{13}\Delta_{\text{mod}}$) for selected fertilizer treatments on the Park Grass Experiment (PGE) and comparison with the 'constant $c_a - c_i$ ' and 'constant c_i/c_a ' response scenarios (Saurer *et al.*, 2004). Filled circles represent the modelled $^{13}\Delta$ values derived from the multiple linear regression model at actual Plant available soil water (PAW_{ref}). Black lines show the estimated changes in $^{13}\Delta$ with increasing atmospheric CO_2 at mean PAW_{ref} (63.6 mm) as derived from the multiple linear regression model. The grey-shaded areas mark the 'constant $c_a - c_i$ ' (upper limit) and 'constant c_i/c_a ' (lower limit, resp. zero line) response scenarios of Saurer *et al.* (2004). Asterisks mark the significance level (***) (< 0.001) of the slope parameters. (b) Long-term trends of intrinsic water-use efficiency (W_i) of selected limed treatments on the PGE. Open circles represent W_i as derived from measured $^{13}\Delta$ values, black lines show the estimated trend in W_i as derived from $^{13}\Delta_{\text{mod}}$ at mean PAW_{ref} (63.6 mm).

effects, biome type (boreal forest trees vs. temperate grassland communities), the presence of particular functional groups, initial conditions and duration of the study.

In the present work the supply of only PK had no differential effect on the CO_2 response in comparison with the N fertilized treatments (*Low N*, *Low N+PK*, *High N+PK*). We believe that this response of the PK treatment was related to the promotion of legumes and associated N fixation. The amount of the estimated biologically fixed N on the PK treatment ($50 \text{ kg ha}^{-1} \text{ a}^{-1}$) was very similar to the moderate N fertilization level ($48 \text{ kg ha}^{-1} \text{ a}^{-1}$). Accordingly, P plus K supply enhanced yield, the Ellenberg N indicator value and NNI of the PK treatment. It is well known from many grassland ecosystem studies that a combination of P

and K fertilizer supply can greatly enhance the abundance and N fixation rate of legumes in P and K poor soils (e.g., Cadisch *et al.*, 1993).

Lime was applied to the studied treatments of the PGE as was required to maintain the soil pH near 7. Conversely, there was no lime treatment in the Betson *et al.* (2007) study. That soil pH (or calcium) could have been a factor is indicated by the divergent CO_2 response of $^{13}\Delta$ in the limed and unlimed *Control* treatments of the PGE: in our previous study (Köhler *et al.*, 2010), the unlimed *Control* (soil pH ~ 5.2) did not show a trend in $^{13}\Delta$, whereas the present limed *Control* (pH ~ 7.2) showed significantly increasing $^{13}\Delta$. Similarly, unfertilized grassland at the Augstmatthorn (a mountain in the Bernese calcareous Alps) exhibited a significant (positive) $^{13}\Delta$ response (Barbosa *et al.*, 2010).

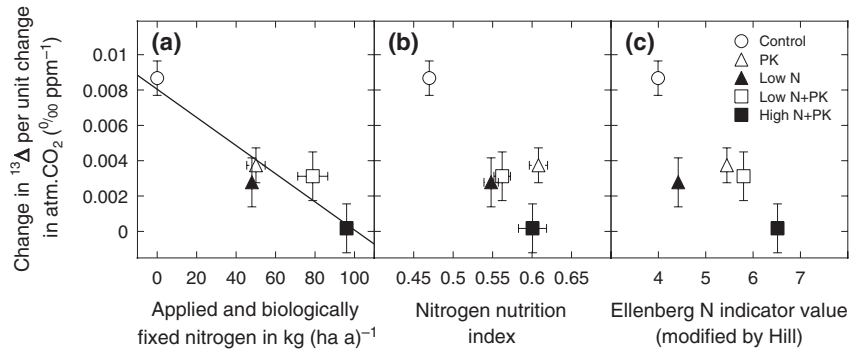


Fig. 3 The relationship between the long-term $^{13}\Delta$ response (shown in Fig. 2a) and (a) N input, (b) N nutrition index (NNI) and (c) Ellenberg N indicator value. Horizontal and vertical errorbars represent the respective standard errors. The black line in (a) is the linear regression line ($y = -0.00008x + 0.00804$, $r^2 = 0.88$, $P < 0.02$).

The species or functional group composition of the community – modified by pH or nutrient availability – could also underlie the $^{13}\Delta$ response. Liming and fertilizers had strong effects on species and functional group composition on the PGE (Silvertown *et al.*, 2006). Plant functional groups, but also species within the same functional group, have been found to differ in their responses to elevated CO_2 (e.g., Reich *et al.*, 2001; Poorter & Navas, 2003; Crous *et al.*, 2010). For instance, the response of g_s to elevated CO_2 is greater in C_3 grasses than in forbs (Ainsworth & Rogers, 2007). Correspondingly, we observed a significant relationship between the $^{13}\Delta$ response and the proportion of grasses in the sward ($P < 0.05$), when the unlimed *Control* treatment from our previous study (Köhler *et al.*, 2010) was included (Fig. 4). This may indicate that the stronger increase in W_i under increasing CO_2 on the fertilized treatments resulted from their greater C_3 grass content and, furthermore, that this enhancement was related to a greater reduction in g_s , rather than to a greater stimulation of A by increasing CO_2 . This interpretation agrees with Jenkinson *et al.* (1994), who found no change in yield – a proxy of A – on different plots of the PGE from 1891 to 1992. The implied relationship between grass content and CO_2 response of $^{13}\Delta$ may also explain the difference between the limed and unlimed *Control* treatments, which differed in the mean contribution (\pm SD) of grasses ($49 \pm 12\%$ vs. $64 \pm 16\%$ respectively).

The duration and starting conditions of the study could also influence the CO_2 response of $^{13}\Delta$ or our ability to detect such a response. The long-term change in $^{13}\Delta$ ranged between nil and 0.7% in the different treatments when CO_2 concentration increased by $86 \mu\text{mol mol}^{-1}$ from 1915 to 2009. These changes accounted for only 10% of the total variation in $^{13}\Delta$ observed in this period. The greater part of the variation ($\sim 44\%$) was related to short-term weather effects on $^{13}\Delta$, generating ‘noise’ hindering the detection of the

climate change-related trend. If the analysis of the long-term change was restricted to the same 32 years long period as the study of Betson *et al.* (2007), we did not detect any treatment effect and the $^{13}\Delta$ responses were closer to the ‘passive response’ scenario. This emphasizes that very long observation series may be needed to ascertain interactive effects of fertilizers and CO_2 on $^{13}\Delta$ resp. W_i .

The starting conditions and successional stage of the experiment also differed between this study and that of Betson *et al.* (2007). Effectively, Betson *et al.* (2007) studied a mono-culture response in years 1–32 of altered N fertilizer supply rates. Conversely, this study investigated the CO_2 response for communities that had previously adapted to different rates of fertilizer supply since 1856, leading to different species compositions. The fertilizer treatments were established at a time when the atmospheric CO_2 level was approximately stable at $280 \mu\text{mol mol}^{-1}$. Accordingly, differences in the successional stage, and related differences in functional group composition (see Table 1 and Fig. 4) may have modified the CO_2 response of $^{13}\Delta$ resp. W_i .

Overall, our data indicate that fertilizer supply enhanced the community-scale CO_2 response of W_i , causing greater relative water savings with high nutrient supply than with no supply. This must result from a distinct effect on the regulation of A and/or g_s . Jenkinson *et al.* (1994) showed that yields have not increased at Park Grass, at least on the unlimed plots. If we accept that yield is a proxy of A , then it seems that A has not changed, and increasing W_i was the result of decreasing g_s . The smallest reduction in g_s would then have occurred on the limed *Control* treatment. A direct causal relationship between N supply and W_i response cannot be established, as the effect may be related indirectly to the effect of N availability on botanical composition, as indicated by the relationship with the percentage contribution of grasses in the swards.

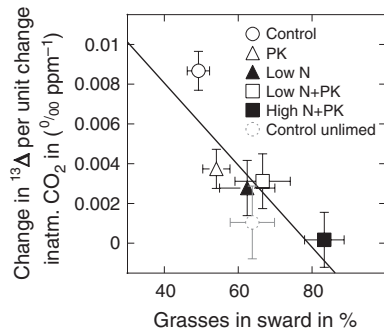


Fig. 4 The relationship between the long-term $^{13}\Delta$ response and the average contribution of grasses in the swards as calculated from botanical separation data from single years during the 1915–1976 period and for each year in the 1991–2000 period. The black line is the linear regression line ($y = -0.0002x + 0.0163$, $r^2 = 0.67$, $P < 0.05$). Horizontal error bars represent the standard deviation of the percentage grass contribution in the swards. Vertical error bars represent the standard error of the slope.

Furthermore, soil pH may affect the botanical composition of grassland swards and recent studies also indicate that not only the amount of N but also the form in which N is supplied (nitrate or ammonium) can affect the CO_2 response (Bloom *et al.*, 2011). This suggests that the effect of N on the plant community response to elevated CO_2 can be complex, emphasizing the need for further studies of the variation in the long-term CO_2 response of W_i at ecosystem level. These would include investigations of the interactions between nutrient availability (taking into account nutrient type, form and amount) and biodiversity, on CO_2 responsiveness of ecosystems.

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References

Ainsworth EA, Rogers A (2007) The response of photosynthesis and stomatal conductance to rising $[\text{CO}_2]$: mechanisms and environmental interactions. *Plant, Cell and Environment*, **30**, 258–270.

Allen RC, Pereira LS, Raes D, Smith M (1998) *Crop Evapotranspiration – Guidelines for computing Crop Water Requirements – FAO Irrigation and Drainage Paper 56*. Food and Agricultural Organization of the United Nations, Rome.

Andreu-Hayles L, Planells O, Gutiérrez E, Muntan E, Helle G, Anchukaitis KJ, Schleser GH (2011) Long tree-ring chronologies reveal 20th century increases in water-use efficiency but no enhancement of tree growth at five Iberian pine forests. *Global Change Biology*, **17**, 2095–2112.

Arnett A, Lloyd J, Šantrůčková H *et al.* (2002) Response of central Siberian Scots pine to soil water deficit and long-term trends in atmospheric CO_2 concentration. *Global Biogeochemical Cycles*, **16**, 1005, doi: 10.1029/2000GB001374.

Avery BW, Catt JA (1995) *The soil at Rothamsted*. Lawes Agricultural Trust, Harpenden, UK.

Barbosa ICR, Köhler IH, Auerswald K, Lüps P, Schnyder H (2010) Last-century changes of alpine grassland water-use efficiency—a reconstruction through carbon isotope analysis of a time-series of *Capra ibex* horns. *Global Change Biology*, **16**, 1171–1180.

Bert D, Leavitt SW, Dupouey JL (1997) Variations of wood $\delta^{13}\text{C}$ and water-use efficiency of *Abies alba* during the last century. *Ecology*, **78**, 1588–1596.

Betson NR, Johannisson C, Lofvenius MO, Grip H, Granstrom A, Hogberg P (2007) Variation in the $\delta^{13}\text{C}$ of foliage of *Pinus sylvestris* L. in relation to climate and additions of nitrogen: analysis of a 32-year chronology. *Global Change Biology*, **13**, 2317–2328.

Betts RA, Boucher O, Collins M *et al.* (2007) Projected increase in continental runoff due to plant responses to increasing carbon dioxide. *Nature*, **448**, 1037–1041.

Bloom A, Asensio J, Randall L, Rachmilevitch S, Cousins A, Carlisle E (2011) CO_2 enrichment inhibits shoot nitrate assimilation in C_3 but not in C_4 plants and slows growth under nitrate in C_3 plants. *Ecology*, **93**, 355–367.

Cadisch G, Sylvester-Bradley R, Boller BC, Nösberger J (1993) Effects of phosphorus and potassium on N_2 fixation (^{15}N -dilution) of field-grown *Centrosema acutifolium* and *C. macrocarpum*. *Field Crops Research*, **31**, 329–340.

Crawley MJ, Johnston AE, Silvertown J *et al.* (2005) Determinants of Species Richness in the Park Grass Experiment. *American Naturalist*, **165**, 179–192.

Crous KY, Reich PB, Hunter MD, Ellsworth DS (2010) Maintenance of leaf N controls the photosynthetic CO_2 response of grassland species exposed to 9 years of free-air CO_2 enrichment. *Global Change Biology*, **16**, 2076–2088.

Diekmann M (2003) Species indicator values as an important tool in applied plant ecology – a review. *Basic and Applied Ecology*, **4**, 493–506.

Dodd M, Silvertown J, McConway K, Potts J, Crawley M (1998) Application of the British National Vegetation Classification to the communities of the Park Grass Experiment through time. *Folia Geobotanica*, **33**, 321–334.

Duquesnay A, Bréda N, Stievenard M, Dupouey JL (1998) Changes of tree-ring $\delta^{13}\text{C}$ and water-use efficiency of beech (*Fagus sylvatica* L.) in north-eastern France during the past century. *Plant, Cell and Environment*, **21**, 565–572.

Ellenberg H, Weber HE, Düll R, Wirth V, Werner W, Paulißen D (1992) Indicator values of plants in Central Europe. *Scripta Geobotanica*, Vol. 18, 2nd edn, pp. 249–253. Verlag Erich Goltze KG, Göttingen.

Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology*, **40**, 503–537.

Feng XH (1999) Trends in intrinsic water-use efficiency of natural trees for the past 100–200 years: a response to atmospheric CO_2 concentration. *Geochimica et Cosmochimica Acta*, **63**, 1891–1903.

Francey RJ, Allison CE, Etheridge DM *et al.* (1999) A 1000-year high precision record of $\delta^{13}\text{C}$ in atmospheric CO_2 . *Tellus*, **51B**, 170–193.

Friedli H, Löffler H, Oeschger H, Siegenthaler U, Stauffer B (1986) Ice core record of the $^{13}\text{C}/^{12}\text{C}$ ratio of atmospheric CO_2 in the past two centuries. *Nature*, **324**, 237–238.

Gagen M, Finsinger W, Wagner-Cremer F *et al.* (2011) Evidence of changing intrinsic water-use efficiency under rising atmospheric CO_2 concentrations in Boreal Fennoscandia from subfossil leaves and tree ring $\delta^{13}\text{C}$ ratios. *Global Change Biology*, **17**, 1064–1072.

Gedney N, Cox PM, Betts RA, Boucher O, Huntingford C, Stott PA (2006) Detection of a direct carbon dioxide effect in continental river runoff records. *Nature*, **439**, 835–838.

Hill MO, Mountford JO, Roy DB, Bunce RGH (1999) *Ellenberg's Indicator Values for British plants. ECOFACT Volume 2 Technical Annex*. Institute of Terrestrial Ecology, Huntingdon.

Højh-Jensen H, Loges R, Jørgensen FV, Vinther FP, Jensen ES (2004) An empirical model for quantification of symbiotic nitrogen fixation in grass-clover mixtures. *Agricultural Systems*, **82**, 181–194.

Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. *Biometrical Journal*, **50**, 346–363.

Jenkinson DS, Potts JM, Perry JN, Barnett V, Coleman K, Johnston AE (1994) Trends in herbage yields over the last century on the Rothamsted long-term Continuous Hay Experiment. *Journal of Agricultural Science*, **122**, 365–374.

- Keeling RF, Piper SC, Bollenbacher AF, Walker JS (2009) Atmospheric CO₂ records from sites in the SIO air sampling network. In: *Trends: A Compendium of Data on Global Change*. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, Tennessee. doi: 10.3334/CDIAC/atg.035. Available at: <http://cdiac.ornl.gov/trends/co2/sio-mlo.html> (accessed 7 November 2011).
- Köhler IH, Poulton PR, Auerswald K, Schnyder H (2010) Intrinsic water-use efficiency of temperate semi-natural grassland has increased since 1857: an analysis of carbon isotope discrimination of herbage from the Park Grass Experiment. *Global Change Biology*, **16**, 1531–1541.
- Lemaire G, Gastal F (1997) N uptake and distribution in plant canopies. In: *Diagnosis of the Nitrogen Status in Crops* (ed. Lemaire G), pp. 3–43. Springer-Verlag, Heidelberg.
- Marshall JD, Monserud RA (1996) Homeostatic gas-exchange parameters inferred from ¹³C/¹²C in tree rings of conifers. *Oecologia*, **105**, 13–21.
- McCarroll D, Loader NJ (2004) Stable isotopes in tree rings. *Quaternary Science Reviews*, **23**, 771–801.
- McCarroll D, Gagen MH, Loader NJ *et al.* (2009) Correction of tree ring stable carbon isotope chronologies for changes in the carbon dioxide content of the atmosphere. *Geochimica et Cosmochimica Acta*, **73**, 1539–1547.
- Peñuelas J, Hunt JM, Ogaya R, Jump AS (2008) Twentieth century changes of tree-ring ¹³C at the southern range-edge of *Fagus sylvatica*: increasing water-use efficiency does not avoid the growth decline induced by warming at low altitudes. *Global Change Biology*, **14**, 1076–1088.
- Peñuelas J, Canadell JG, Ogaya R (2011) Increased water-use efficiency during the 20th century did not translate into enhanced tree growth. *Global Ecology and Biogeography*, **20**, 597–608.
- Poorter H, Navas ML (2003) Plant growth and competition at elevated CO₂: on winners, losers and functional groups. *New Phytologist*, **157**, 175–198.
- R Development Core Team (2012) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. Available at: <http://www.R-project.org> (accessed 12 March 2012).
- ERA (2011) *Rothamsted Electronic Archive*. Datasets: PARKCOMP, PARKCOMPIC, PARKPARTCOMP, ROTHMET. Available at: <http://www.era.rothamsted.ac.uk> (accessed 29 November 2011).
- Reich PB, Tilman D, Craine J *et al.* (2001) Do species and functional groups differ in acquisition and use of C, N and water under varying atmospheric CO₂ and N availability regimes? A field test with 16 grassland species. *New Phytologist*, **150**, 435–448.
- Saurer M, Siegelwold RTW, Schweingruber FH (2004) Carbon isotope discrimination indicates improving water-use efficiency of trees in northern Eurasia over the last 100 years. *Global Change Biology*, **19**, 2109–2120.
- Schnyder H, Schwertl M, Auerswald K, Schäufele R (2006) Hair of grazing cattle provides an integrated measure of the effects of site conditions and interannual weather variability on ¹³C of temperate humid grassland. *Global Change Biology*, **12**, 1315–1329.
- Silvertown J, Poulton P, Johnston E, Edwards G, Heard M, Biss PM (2006) The Park Grass Experiment 1856–2006: its contribution to ecology. *Journal of Ecology*, **94**, 801–814.
- Stitt M, Krapp A (1999) The interaction between elevated carbon dioxide and nitrogen nutrition: the physiological and molecular background. *Plant, Cell and Environment*, **22**, 583–621.
- Waterhouse JS, Switsur VR, Barker AC, Carter AHC, Hemming DL, Loader NJ, Robertson I (2004) Northern European trees show a progressively diminishing response to increasing atmospheric carbon dioxide concentrations. *Quaternary Science Reviews*, **23**, 803–810.
- White JWC, Vaughn BH (2011) Stable Isotopic Composition of Atmospheric Carbon Dioxide (¹³C and ¹⁸O) from the NOAA ESRL Carbon Cycle Cooperative Global Air Sampling Network, 1990–2010, Version: 2011-11-08. University of Colorado, Institute of Arctic and Alpine Research (INSTAAR). Available at: <ftp://ftp.cmdl.noaa.gov/ccg/co2c13/flask/event/> (accessed 7 November 2011).