

Carbon balance and water use efficiency of frequently cut *Lolium perenne* L. swards at elevated carbon dioxide

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Abstract

The impact of doubled atmospheric [CO₂] on the carbon balance of regularly cut *Lolium perenne* L. swards was studied for two years under semi-field conditions in the Wageningen Rhizolab. CO₂ and H₂O vapour exchange rates of the swards were measured continuously for two years in transparent enclosures. The light utilization efficiencies of the swards ranged between 1.5 g CO₂ MJ⁻¹ global radiation (high light, ambient [CO₂]) and 2.8 g CO₂ MJ⁻¹ (low light, doubled [CO₂]). The above-ground net primary productivity (NPP) in the enclosures was greater by 29% in 1994 and 43% in 1995 in the doubled [CO₂] treatments, but only 20% and 25% more carbon was recovered in the periodical cuts. Thus, NPP increased significantly more than did the harvested above-ground biomass. The positive [CO₂] effect on net carbon assimilation is therefore associated with a preferential allocation of extra carbon to the roots and soil.

In addition to higher canopy photosynthesis and leaf elongation rates, a small part of the positive [CO₂] effects on NPP could be attributed to a decrease of the specific respiration of the shoots. On a canopy basis however, respiration was equal or slightly higher at doubled [CO₂] due to the higher amount of standing biomass.

Comparison of NPP and carbon recovered in different harvests showed that allocation to roots and soil was highest in spring, it was low in early summer and increased again in late summer and autumn.

The total gross amount of carbon partitioned to the roots and soil during the two year period was 57% more at doubled [CO₂]. The total amount of carbon that was sequestered in the soil after subtraction of the respiratory losses was 458 g m⁻² and 779 g m⁻² in the ambient and doubled [CO₂] treatments, respectively.

The average water use efficiency (WUE) of the swards was increased by a factor 1.5 at doubled [CO₂]. Both WUE and its positive interaction with [CO₂] varied between years and were positively correlated with global irradiance. At doubled [CO₂], the higher WUE was fully compensated for by a higher leaf area index. Therefore, total transpiration on a canopy basis was equal for the ambient and the doubled [CO₂] concentrations in both years.

Keywords: CO₂, climate change, grassland, light utilization efficiency, *Lolium perenne*, photosynthesis, respiration, transpiration, water use efficiency

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Introduction

The CO₂ exchange rates between atmosphere and biosphere on a global scale are 20 times greater than anthropogenic CO₂ releases into the atmosphere (Gifford 1994).

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Therefore, a small relative change in the gross primary productivity on the one hand and respiratory processes on the other, may influence the trend of steadily increasing atmospheric [CO₂]. At present, carbon sinks are converted into carbon sources mainly as a result of deforestation (Goudriaan 1992). The short-term consequence is a release of carbon from the global pool of plant biomass, while the long-term effects are largely unknown and will depend on

the vegetation to develop after forest clearance. Half of the deforested area is presently being converted into agricultural land and half into grasslands, steadily increasing the global grassland area at a rate of 6 Mha y^{-1} . The carbon density in grassland soils is about twice that in tropical forest soils and even more than twice that in agricultural soils. On a global scale, grassland soils contain about 22% of the total soil carbon (Lashof 1989). Thus grasslands, especially the soils, can act as a sink for the carbon released into the atmosphere by human activities, provided that the carbon sequestered remains in the soil for decades and is not rapidly released. How realistic is this idea? Can a potential increase in net primary productivity be expected to equilibrate at a higher level, or is it a transient response that will be counterbalanced by steadily increasing rates of plant and soil respiration? It is now well known that vegetation becomes a greater sink for carbon at elevated $[CO_2]$ due to the CO_2 fertilization effect (Kimball 1983; Goudriaan & Ketner 1984; Long 1991; Parton *et al.* 1995), but experimental results are not as conclusive when examining heterotrophic respiration during litter decomposition (Ryle *et al.* 1992; Gorissen *et al.* 1995; Körner & Miglietta 1994). In addition, interactions with temperature, nutrient and water availability remain a point of debate (Gifford 1994; Zak *et al.* 1993; Gorissen *et al.* 1995; Grashoff *et al.* 1995). While in the most simple hypothesis, respiration increases proportionally to photosynthesis, the actual situation could differ from this simple model in two opposing directions:

1 The increase in gross photosynthesis is not translated into dry matter accumulation, but is compensated for by negative feedback on the photosynthetic rate, resulting in the accumulation of non-structural carbohydrates (Stitt 1991; Körner & Miglietta 1994). Non-structural carbohydrates will be rapidly respired after the plant dies so that the contribution to carbon sequestering in the soil will be small.

2 The increase of gross photosynthesis is not translated into increased respiration or decomposition, so that the resulting dry matter accumulation increase is more than proportional to the increase in $[CO_2]$.

Increasing concentrations of atmospheric CO_2 will influence plant-water relations. Elevated $[CO_2]$ increases the water-use efficiency and a beneficial effect may be expected when water resources are limiting (Gifford 1994). Soil water could then be preserved and the productive growth stage prolonged (Bremer *et al.* 1996). However, the validity of this statement might be questioned when the stored water is more rapidly depleted due to higher growth rates and the greater leaf area for transpiration at elevated $[CO_2]$.

Our contribution to the debate on carbon sequestering in vegetation and soil and on changes in the water use

efficiency of vegetation at elevated $[CO_2]$ is based upon a long-term experiment. It was performed to quantify photosynthesis, dark respiration, transpiration and carbon allocation to shoots, stubble and roots in grass monocultures at doubled and ambient $[CO_2]$ at an optimum nutrient and water supply. The global picture of course, is composed of many different plant communities in which environmental influences are more complex than in highly fertilized and irrigated monocultures of ryegrass. However, the basic principles of grassland carbon balance in long-term experiments can be probably best comprehended by using an experimental set-up with as few interacting factors as possible. The results of this study have to be matched to ecological studies to answer the question: can we expect that grasslands will sequester more carbon in an elevated $[CO_2]$ world and if so, will water become the limiting factor to primary production?

Material and methods

A two-year experiment was begun in October 1993 with *Lolium perenne* L. (cv Preference). Swards were grown from seed in the Wageningen Rhizolab (Van de Geijn *et al.* 1994) in translucent polycarbonate enclosures at 350- and 700 vpm CO_2 in two replicates and were surrounded by swards to prevent border effects. The soil compartments were 1.25 m \times 1.25 m and 2 m deep and were filled with a repacked loamy clay soil, with 4.7% organic matter and a pH of 7.0. Each sward was fitted with a separate drip irrigation system and at every 15 cm below the soil surface, sensors were installed to measure soil moisture status, electrical conductivity and temperature. Each plot and its surrounding borders were irrigated, equivalent to 10 mm rainfall, when the soil water status in one of the layers in the soil profile dropped below a volumetric moisture content of 27%. The temperature in the enclosures was controlled by air conditioners and followed the measured outside air temperature. Global irradiance was measured by Kipp solarimeters installed at an altitude of three meter next to the experimental plots. The transmission coefficient of the polycarbonate enclosures was 79%. Photosynthesis, transpiration and dark respiration of the swards were monitored at six minute intervals in all compartments. The gas exchange rates in the enclosures were calculated from the differences between partial pressures of the respective gases in ingoing and outgoing air multiplied by the air flow rates. The supply of fresh air in each above ground compartment was 50 m³ h⁻¹ and internal circulation rate was 800 m³ h⁻¹. The CO_2 produced in the soil compartment could not enter the enclosures due to the existing over-pressure. An air drain at a depth of 15 cm collected the downward air flow of about 30 m³ h⁻¹, which was analysed to determine soil and root respiration.

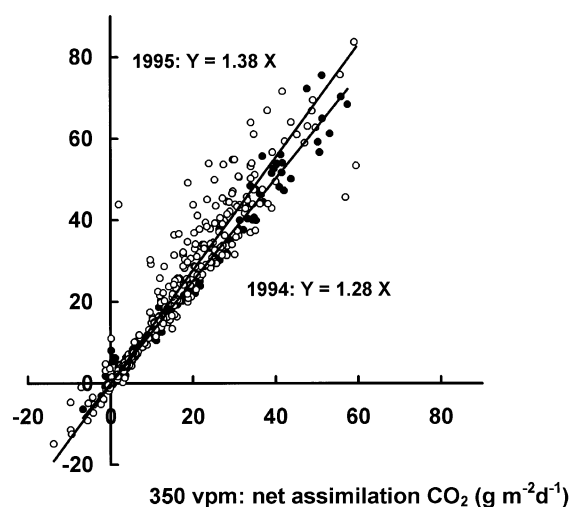
700 vpm: net assimilation CO₂ (g m⁻²d⁻¹)

Fig. 1 Regression lines of daily net assimilation rates at ambient and doubled [CO₂] concentrations in 1994 (closed symbols) and 1995 (open symbols).

Condensed water in the air conditioners was automatically weighed every hour and the cumulative amount was used to estimate the total measured evapo-transpiration rates.

The swards were cut 10 times per year to 5 cm high at intervals of 20–24 days, after which potassium nitrate (8 g N m⁻²) was supplied. The number of tillers was then counted, and the leaf elongation rates were determined 6 days after cutting from measurements of leaf length. At the end of 1994, in spring 1995 and at the last harvest in autumn 1995, soil samples (4 per soil compartment) were taken as a 95 cm deep soil core. The roots were isolated and analysed for dry weight and carbon content. At the end of the experiment the stubble was harvested and analysed analogously.

The results were analysed with ANOVA statistical analyses.

Results

Photosynthesis

The CO₂ gas exchange rates of the shoots in the enclosures, measured at six minute intervals, were integrated to daily totals. The CO₂ released by root respiration did not enter into these data, because it could not diffuse from the soil due to the over-pressure in the enclosures. Figure 1 shows the integrated daily net CO₂ assimilation rates at 700 vpm plotted against those measured at 350 vpm. The stimulation at doubled [CO₂] was estimated by linear regression analysis. The CO₂ response at higher assimilation rates was greater than expected from the linear trend, especially

Table 1 Carbon fluxes at 350 or 700 vpm [CO₂] derived from gas exchange measurements.

	350 vpm CO ₂	700 vpm CO ₂	increase (%)
Net Primary Productivity (g C m ⁻² y ⁻¹)			
1994	1198	1540	29**
1995	1266	1815	43*
Total	2464	3355	36***
Dark respiration of shoot (g C m ⁻² y ⁻¹)			
1994	-224	-228	2 (ns)
1995	-177	-226	28**
Total	-401	-454	13*
Gross Primary Productivity (g C m ⁻² y ⁻¹)			
1994	1646	1996	21*
1995	1620	2267	40*
Total	3266	4263	31***

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

in 1995. Apparently, the CO₂ effect tended to increase from low to high assimilation rates, or in fact from low to high irradiances. The CO₂ assimilation rates and the CO₂ response to doubled [CO₂] were higher in 1995 than in 1994 ($P < 0.01$, Table 1). In the first place, this shows that the swards did not acclimatise to doubled [CO₂] during the first year. Secondly, the higher stimulation during 1995 is in agreement with the higher irradiance sum in that year (Fig. 2b). Irradiance and temperature are closely linked and although the average temperatures were about equal in 1994 and 1995 (Table 3), we could not distinguish between their separate influences. Within the two years, irradiance and temperature interactions with [CO₂] were reflected in the seasonal variation. There was no [CO₂] stimulation in winter, a 20% stimulation in spring, a 50–90% in summer and 0–30% in autumn. The relationships between average daily NPP and the average global irradiance inside the enclosures for all harvests at doubled and ambient [CO₂] are shown in Fig. 2a. The light utilization coefficient (LUE; g CO₂ MJ⁻¹ incident global irradiance) was derived from assimilated CO₂ in each period between harvests divided by the corresponding irradiance sum. For low irradiance (5 MJ m⁻²), LUE ranged from 2.2 g MJ⁻¹ (ambient [CO₂]) to 2.8 g MJ⁻¹ (doubled [CO₂]). At a high irradiance sum (15 MJ m⁻²), the LUE ranged from 1.5 g MJ⁻¹ (ambient [CO₂]) to 2.2 g MJ⁻¹ (doubled [CO₂]).

Respiration

Like photosynthesis, shoot respiration also contains a biomass component and a mass-independent or specific

Table 2 Harvested dry weights, Leaf Area Index (LAI), Specific Leaf Area (SLA) and tiller number at the harvest dates. Treatments were 350 vpm CO₂ and 700 vpm CO₂.

Year	Date	Harvested dry weight (g m ⁻²)		LAI		SLA (m ² kg ⁻¹)		Tiller (×10 ³)	
		350	700	350	700	350	700	350	700
1994	6 Apr	173	189	4.94	5.00	28.60	26.50	10.16	10.90
	4 May	245	272	7.84	8.58	32.00	31.50	11.59	12.90
	25 May	256	320	9.12	10.66	35.60	33.30	11.03	12.15
	21 Jun	142	160	3.83	4.38	27.00	27.40	7.19	7.41
	7 Jul	196	251	5.10	5.81	26.00	23.10	7.60	8.30
	27 Jul	206	272	4.38	5.55	21.30	20.40	5.09	6.23
	23 Aug	130	152	2.51	2.70	19.30	17.80	5.96	6.16
	20 Sep	144	169	3.23	3.64	22.40	21.50	5.09	5.53
	26 Oct	132	140	2.40	2.11	18.30	15.10	4.60	4.88
	29 Nov	58	52	1.32	1.14	23.10	21.90	5.35	5.80
1995	13 Apr	295	267	4.94	3.72	16.80	13.95	10.57	11.77
	3 May	178	177	3.86	3.29	21.72	18.59	11.56	12.82
	23 May	182	226	3.37	3.59	18.51	15.88	10.58	13.10
	14 Jun	195	234	3.23	3.88	16.58	16.60	7.91	10.88
	5 Jul	216	292	3.48	4.60	16.05	15.75	7.65	9.94
	26 Jul	178	233	3.60	4.52	20.31	19.45	6.57	9.85
	16 Aug	140	212	2.71	8.79	19.40	17.90	7.89	10.50
	6 Sep	134	194	2.56	3.64	19.18	18.70	8.54	10.91
	27 Sep	109	152	3.12	4.09	28.76	27.06	7.37	9.59
	31 Oct	142	182	3.45	4.06	24.37	22.33	7.54	9.74

component, which is related to the chemical composition of the biomass (Amthor 1991). The amount of biomass on one hand and the specific respiration rate on the other, determine the occurrence of elevated or reduced respiration of the canopy at doubled [CO₂] (Schapendonk & Goudriaan 1996). In 1994, [CO₂]-related increase of biomass and a decreased specific respiration led to similar shoot respiration rates. In 1995, however, the effect of a larger standing biomass prevailed over the lower mass-independent respiration, and therefore total shoot respiration was higher at doubled [CO₂] (Fig. 3).

Carbon balance

The net shoot carbon uptake is defined here as net primary productivity (NPP) and in fact equals the net assimilated CO₂ multiplied by the ratios of the molecular weights of CO₂ and carbon: 12/44. The NPP contains both the photosynthesis rate (+) and the respiration rate of the shoot (-). In order to discriminate between these processes, we estimated the effect on gross primary productivity (GPP) by adding the respired carbon to the NPP. We assumed respiration rates to be equal for day and night. Daily total shoot respiration is thus estimated to be twice the dark shoot respiration.

Table 1 and Fig. 4 summarize the measured net carbon fluxes in each year and the totals for the swards in the

two subsequent years. Both the NPP and the stimulation by doubled [CO₂] were higher in 1995 than in 1994 ($P < 0.01$, Table 1), which is in agreement with the higher irradiance observed in 1995. Comparison of NPP (Table 1) with the carbon harvested in the cuts (Table 4a) reveals that considerable fractions of the NPP were not recovered in the harvests, 40% at ambient and 46% at doubled [CO₂]. Thus doubled [CO₂] did not only stimulate GPP and NPP, but also led to a higher, selective flow of carbon to the roots, stubble and the soil. Of the NPP fractions accumulated over the two years, 983 and 1539 g of carbon were allocated to the roots stubble and soil for the ambient and the doubled [CO₂] treatment, respectively, (Table 4b). The roots contained only 66 and 123 g C at the end of the experiment (Table 4a). It remains unknown how much carbon was allocated to the stubble from the start of the observations in 1994. Because the stubble contains mainly stems and leaf sheets, we made an estimation based on the number of tillers. An estimated 10,000 tillers m⁻² in spring 1994 varied during the years and reached a value of 8000 for ambient and 10,000 for doubled [CO₂] in the autumn of 1995 (Table 2). Thus, the stubble biomass was in steady-state and did not increase during the experiment. The carbon released by stubble turn-over is assumed to have become part of the carbon fraction allocated to the soil and root compartment.

Carbon partitioning between above- and below-ground

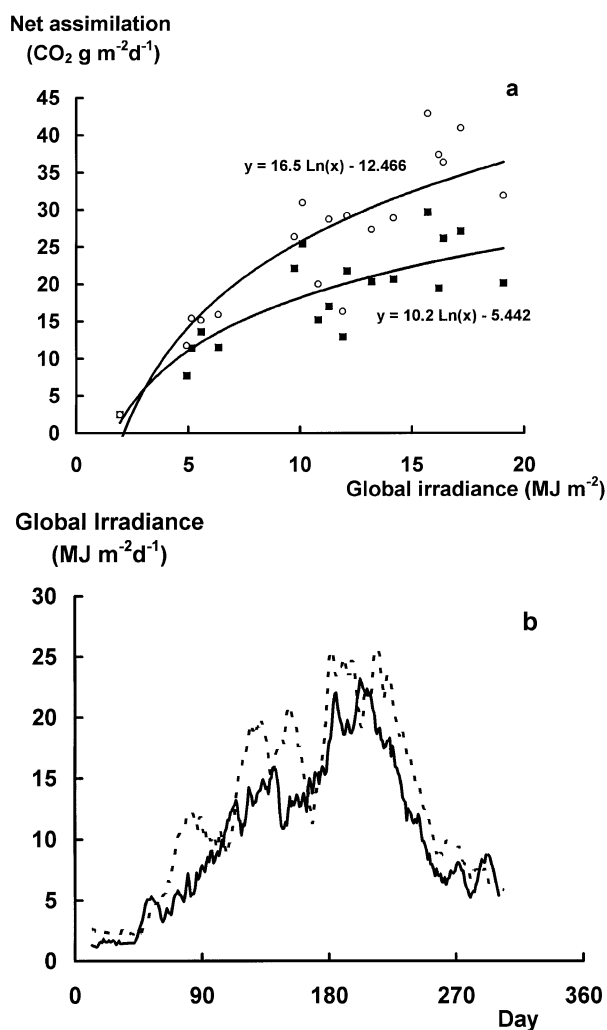


Fig. 2 (a) Average net assimilation rates at ambient [CO₂] (closed squares) and at doubled [CO₂] (open circles) between subsequent cuts plotted as a function of the average daily global irradiance during those periods. (b) Time course of global irradiance in 1994 (continuous lines) and 1995 (broken lines).

Table 3 Integrated outside global radiation (MJ m⁻²) and average temperatures over the time periods between julian Day 60 and 300.

	Global radiation	Temperature (°C)
1994	2869	14.2
1995	3492	14.7

plant parts was not constant throughout the year (Fig. 5a, b). The amount of carbon in subsequent harvests revealed that in early spring 60–70% of the NPP was allocated to stubble and roots. This was reduced to 20–40% in late spring, but increased again later in the year. The high degree of partitioning to the roots in early spring is in

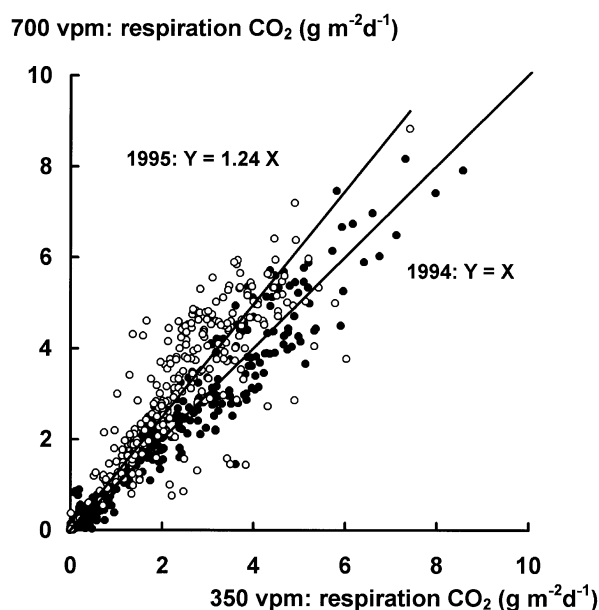


Fig. 3 Regression lines of daily dark respiration rates at ambient and doubled [CO₂] in 1994 (closed symbols) and 1995 (open symbols).

good agreement with the observed increase in live roots in the spring of 1995 (Table 4a).

Water use efficiency (WUE)

WUE for subsequent periods between cuts is shown in Fig. 6. In 1994 and 1995, the WUE in the doubled [CO₂] treatments was significantly higher than at ambient [CO₂]. Similar to the photosynthesis responses there was no acclimation for WUE. The increase in WUE was not a short-term transient response, but was constant during this experiment. Averaged over two years, the WUE was 0.013 g CO₂ g⁻¹ H₂O at ambient [CO₂] and 0.019 g CO₂ g⁻¹ H₂O at doubled [CO₂]. The WUE in summer is much lower than in winter due to the higher vapour pressure deficit of the air and the fact that the photosynthesis is more often light saturated, whereas transpiration increases almost linearly with irradiances. Indeed, we found a strong decline of the WUE in both summers. Stomatal closure at high irradiance will reduce transpiration more than photosynthesis, which means that elevated [CO₂] will have a greater effect on the WUE in summer than in winter. Indeed, we found that the relative effects of doubled CO₂ were small in early spring and autumn and were maximum in summer. The effect of [CO₂] on WUE is apparently highest at high irradiance.

The transpired amounts of water in all harvest periods are depicted in Fig. 7. The amounts were nearly equal for the ambient and the doubled [CO₂] treatments. Since it is well established that there were differences in WUE,

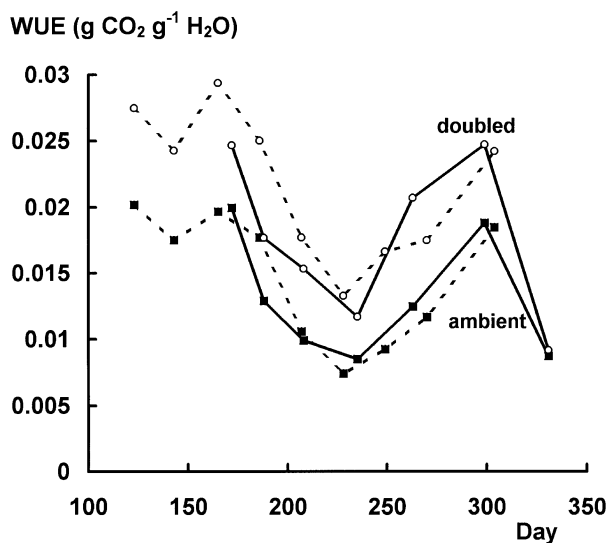


Fig. 6 Water use efficiencies ($\text{g CO}_2/\text{g H}_2\text{O}$) of swards in periods between subsequent cuts in 1994 (continuous lines) and 1995 (broken lines). The doubled $[\text{CO}_2]$ treatments are indicated by open circles and the ambient $[\text{CO}_2]$ treatments by closed squares.

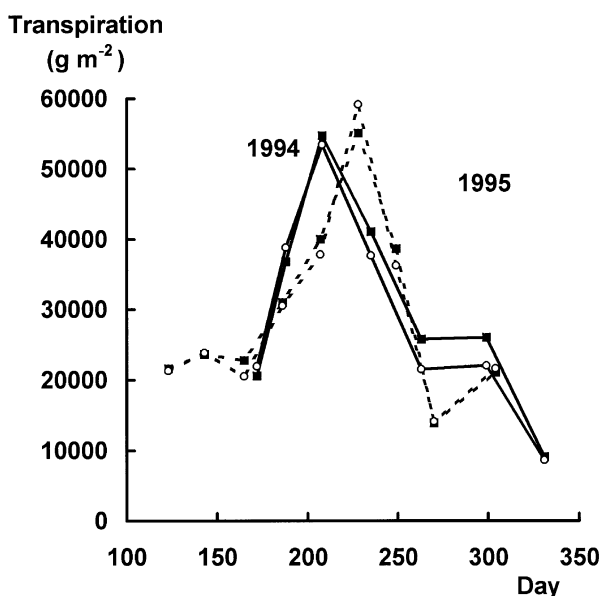


Fig. 7 Total transpiration (g m^{-2}) of swards in periods between cuts in 1994 (continuous lines) and 1995 (broken lines). The doubled $[\text{CO}_2]$ treatments are indicated by open circles and the ambient $[\text{CO}_2]$ treatments by closed squares.

Table 5 Total Leaf Area Duration (LAD) in days, between day 100 and the days of the last harvests.

	350 vpm CO ₂	700 vpm CO ₂	increase (%)
1994	605 d	656 d	8.4
1995	429 d	497 d	15.8

ance in 1995 fully compensated for the lower LAD in that year.

Comparison of $[\text{CO}_2]$ treatments confirms that transpiration on a canopy basis is a very conservative attribute. The higher LADs at doubled $[\text{CO}_2]$ did not enhance transpiration due to the higher WUE induced by the doubled $[\text{CO}_2]$ treatment.

Discussion

The average net carbon uptake in highly fertilized and well irrigated grasslands was stimulated by 36% under doubled $[\text{CO}_2]$, which is within the range established for a great variety of species (Kimball 1983). The stimulation of carbon assimilation at doubled $[\text{CO}_2]$ remained over the two seasons of exposure. Contrasting results have been described for acclimation of photosynthesis at elevated $[\text{CO}_2]$. A very rapid acclimation of natural vegetation was found by Grulke *et al.* (1990) for tundra vegetation and by Wolfenden & Diggle (1995) for upland grassland communities, but no acclimation was found after several seasons for a temperate wetland (Drake & Leadley 1991). It is difficult to isolate processes in complex natural systems, but photosynthetic acclimation as a consequence of sink limitation is undoubtedly a major controlling factor, especially in natural ecosystems (Stitt 1991; Körner & Miglietta 1994; Nijs *et al.* 1992). Conditions for low sink activity are low temperature, shortage of water and nutrients. These are common conditions in natural ecosystems, but often so in agricultural systems (Körner & Miglietta 1994; Grashoff *et al.* 1995; Lutz & Gifford 1995). It is unlikely that elevated $[\text{CO}_2]$ will act directly by stimulating the photosynthesis in sink-limited natural ecosystems (Wolfenden & Diggle 1995), but more likely operates through other plant reactions that have a positive effect on nutrient and water availability or requirements (Owensby *et al.* 1997, this issue). However, it is not possible to predict these repercussions from basic principles and to compare them with primary responses of photosynthesis and respiration. Therefore, we cannot directly extrapolate our results to natural ecosystems.

Interaction with defoliation.

During 4–6 days after cutting, the newly emerging leaves acted as a net sink for stored carbon instead of a source. The respiration rate was higher than photosynthesis, which resulted in a negative NPP. During that period $[\text{CO}_2]$ had no effect on NPP, despite the fact that leaf elongation rates were higher at doubled $[\text{CO}_2]$ (Schapendonk *et al.* 1996). After the first 4–6 days, the NPP became positive and responded to $[\text{CO}_2]$ again (not shown). This has obvious consequences for the $[\text{CO}_2]$ response of different types of grassland. Frequent defoli-

ation by grazing and herbivory will lead to prolonged periods of negative NPP with negligible $[\text{CO}_2]$ responses.

Environmental factors and LAI development

Despite the interaction and strong coupling between light and temperature, there is evidence that temperature is an important factor when it comes to the CO_2 effect on net assimilation (Nijs *et al.* 1992; Grashoff *et al.* 1995). We were not able to separate irradiance and temperature but the variability around the fitted curves during single harvest intervals (Fig. 2a) may be due to temperature effects. Doubling the $[\text{CO}_2]$ increased LUE relatively more at high than at low irradiance (Fig. 2a). In addition to the direct effects on the photosynthetic efficiency, doubled $[\text{CO}_2]$ stimulated the photosynthetic rates of the canopy by increasing the light interception by a higher LAI. The higher LAI not only resulted from higher assimilation rates, but also from more rapid leaf elongation in the first week after cutting when regrowth depends on the remobilization of stored carbon (Schapendonk *et al.* 1996). We also observed a beneficial effect of doubled $[\text{CO}_2]$ on tillering, mainly in the second year (Table 2). This is not surprising because the correlation between yield and tillering is very strong (Schapendonk *et al.* 1990; van Loo *et al.* 1992). The relatively higher tillering and leaf elongation rates under doubled $[\text{CO}_2]$ resulted in a faster development of the LAI after cutting, which amplified the direct $[\text{CO}_2]$ effects on leaf photosynthesis.

Shoot and soil respiration

Whereas the average NPP was increased by 36% under doubled $[\text{CO}_2]$, average shoot respiration only increased by 13%. On the basis of the 22.5% higher shoot biomass, respiration was anticipated to increase proportionally. On the other hand, specific respiration may be lower (Amthor 1995), related to lower costs for the synthesis of structural biomass and for maintenance processes. Because production costs of nitrogen compounds are relatively high, the expected low respiratory costs were in agreement with the observed low average nitrogen content of 3.6% at elevated $[\text{CO}_2]$ compared to 4.8% at ambient $[\text{CO}_2]$.

Respiration of some of the carbon allocated to the soil and roots is a crucial factor in the total balance. When respiratory activities of soils and roots are neglected, the gross accumulation of carbon in the soil is 57% higher at doubled $[\text{CO}_2]$. But how much of this amount will be respired? There is evidence that soil carbon is rapidly mineralized under ambient $[\text{CO}_2]$, until a stable fraction of 40–60% remains after 30 days (Bremer & Kuikman 1994). If the decomposition rate of organic compounds in the soil at doubled $[\text{CO}_2]$ were higher, the net $[\text{CO}_2]$ effect on carbon sequestering in the soil would

be cancelled by an increase in respiration. Decomposition studies however, tend to show the opposite. The turnover of dead root material of *Lolium perenne* L. was found to be slower under elevated $[\text{CO}_2]$ (Gorissen 1996). In agreement with these results, the cumulative soil respiration in the soil compartments appeared to be 10% lower at doubled $[\text{CO}_2]$ (Schapendonk & Goudriaan 1996). These data, however, should be interpreted with caution because they were obtained from measurements in which the air from the enclosures was forced through the upper soil layer into a drain to the infra-red gas analyser. The CO_2 concentration in the upper soil layer was therefore 350 vpm or 700 vpm which is much lower than concentrations usually found in undisturbed soils. The low CO_2 concentration and the continuous oxidizing stream of air passing through the soil might have increased microbial and root respiratory processes. Although the absolute levels of respiration may be affected in this way, the relative effect of elevated CO_2 on respiration is more likely related to changes in the chemical composition of the roots and microbial biomass, which is probably not biased by the gaseous composition in the soil. We therefore assumed that on average 50% of the soil carbon was stabilized for a longer time span and that root and soil respiration were 10% lower at doubled CO_2 . We then obtained the overall view shown in Fig. 4. It implies that after 2 years the amount of carbon increased by 458 g m^{-2} at ambient and 779 g m^{-2} at doubled CO_2 . These findings agree with the results of Van Ginkel *et al.* (1997), who found an annual carbon input of 166 g m^{-2} and 272 g m^{-2} for ambient and doubled CO_2 , respectively. The amount of carbon in the soil profile of the Rhizolab down to a depth of one meter was about 32000 g m^{-2} . Thus, the amount of carbon sequestered in the soil profile was about 1.4% and 2.4% of the existing carbon pool at ambient and elevated $[\text{CO}_2]$, respectively.

WUE

In what direction and to what extent does water supply interact with the CO_2 effects on NPP and carbon sequestering? Long-term measurements of transpiration combined with net CO_2 uptake are scarce, although immediate responses are expected to be larger than long-term responses (Morison 1993; Santrucek & Sage 1996). A shortage of water on the short-term leads to a decrease in stomatal conductance and a transient reduction of the internal $[\text{CO}_2]$ (Schapendonk *et al.* 1989). The internal $[\text{CO}_2]$ seems to equilibrate after a few days to about the same value as prior to the water shortage (Wong *et al.* 1979, 1985). Thus stomatal aperture adapts to the mesophyll limitation and both show a correlated response to water stress. The $[\text{CO}_2]$ ratio (c_i/c_a) under water stress conditions and the proportion of gas phase resistance to

total resistance then remains constant. Photosynthesis and transpiration then are equally reduced and therefore, WUE is constant.

At doubled [CO₂] the average WUE was almost 50% higher than at ambient [CO₂], which is similar to results found in other grassland ecosystems (Bremer *et al.* 1996). Acclimation of photosynthesis to elevated [CO₂] implicitly decreases WUE. Vice versa, no acclimation of photosynthesis will lead to a stable WUE. Indeed, we found no evidence for acclimation of the CO₂ response, neither for photosynthesis nor for transpiration. The coupling between photosynthetic acclimation and stomatal acclimation however, is not always fixed. Temperature for instance, causes variable responses between species (Santrucek & Sage 1996).

In order to establish a lower water consumption, it is essential that the higher leaf WUE should not be compensated by a CO₂-induced increase in sward surface area (Kerstiens *et al.* 1995). We observed that these factors did in fact compensate. An 8–16% increase in LAD (Table 5) at doubled [CO₂] was fully compensated for by a 50% increase in WUE. The relative increase in LAD is apparently more important for the balance of transpiration changes than the relative increase in WUE. When the CO₂ effect on LAI is absent (Lutz & Gifford 1995), transpiration will only be determined by WUE, but even small increases of LAD, as the 8–16% increase found for our system, will cancel the water saving consequences.

The WUE declined in summer because the net CO₂ assimilation became light saturated, while transpiration constantly increased with increasing irradiance. Total transpiration rates per cutting interval however, were equal at largely varying irradiances for the ambient and the doubled [CO₂] (Fig. 7). This is not a coincidence, but due to the effects of irradiance and (CO₂) on both LAI and WUE. For instance, higher irradiance enlarged the CO₂ effect on LAI and thus on transpiration, but it also enhanced the [CO₂] effect on WUE by a decreased transpiration. This mechanistic feed-back may annul the [CO₂] effect on transpiration independent of the irradiance as shown in Fig. 7.

In conclusion, carbon sequestering in grassland soils at doubled [CO₂] over two years was about twice the amount sequestered at present ambient [CO₂]. This was due to a higher NPP, a preferential allocation of carbon to roots and soil and a reduced decomposition rate of organic compounds in the soil under doubled [CO₂]. The observed doubling of carbon sequestered in the soil under doubled [CO₂] is probably the upper limit. The NPP will respond less strongly in limited-supply systems. However, the direction of carbon allocation under those conditions may be directed even more to the roots and soil under elevated CO₂ (Van Ginkel *et al.* 1997). With respect to the limited water supply, our observations lead

to the conclusion that the increase in NPP and soil carbon sequestering did not require more water because the CO₂ effects on LAI and on WUE were compensating over a wide range of varying irradiances.

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References

- Amthor JS (1991) Respiration in a future, higher CO₂ world. *Plant, Cell and Environment*, **14**, 13–20.
- Amthor JS (1995) Terrestrial higher-plant response to increasing atmospheric [CO₂] in relation to the global carbon cycle. *Global Change Biology*, **1**, 243–274.
- Bremer E, Kuikman P (1994) Microbial utilisation of ¹⁴C[U]Glucose in soil is affected by the amount and timing of Glucose additions. *Soil Biology and Biochemistry*, **4**, 511–517.
- Bremer DJ, Ham JM, Owensby CE (1996) Effects of elevated atmospheric carbon dioxide and open-top chambers on transpiration in a tall grass prairie. *Journal of Environmental Quality*, **25**, 691–701.
- Drake BG, Leadley PW (1991) Canopy photosynthesis of crops and native plant communities exposed to long-term elevated CO₂. *Plant, Cell and Environment* **14** 853–860.
- Gifford RM (1994) The global carbon cycle: a viewpoint on the missing sink. *Australian Journal of Plant Physiology*, **21**, 1–15.
- Gorissen A (1996) Elevated CO₂ evokes quantitative and qualitative changes in carbon dynamics in a plant/soil system: mechanism and implications. *Plant and Soil*, **187**, 289–298.
- Gorissen A, Van Ginkel JH, Keurentjes JJB, Van Veen JA (1995) Grass root decomposition is retarded when grass has been grown under elevated CO₂. *Soil Biology and Biochemistry*, **27**, 117–120.
- Goudriaan J (1992) Biosphere structure, carbon sequestering potential and the atmospheric ¹⁴C carbon record. *Journal of Experimental Botany*, **43**, 1111–1119.
- Goudriaan J, Ketner P (1984) A simulation study for the global carbon cycle, including man's impact on the biosphere. *Climate Change*, **6**, 167–192.
- Grashoff C, Dijkstra P, Nonhebel S, Schapendonk AHCM, Van de Geijn SC (1995) Effects of climate change on productivity of cereals and legumes; model evaluation of observed year-to-year variability of the CO₂ response. *Global Change Biology*, **1**, 417–428.
- Grulke NE, Riechers GH, Oechel WC, Hjelm U, Jaeger C (1990) Carbon balance in tussock tundra under ambient and elevated atmospheric CO₂. *Oecologia*, **83**, 485–494.
- Kerstiens G, Townend J, Heath J, Mansfield TA (1995) Effects of water and nutrient availability on physiological responses of woody species to elevated CO₂. *Forestry*, **68**, 303–315.

- Kimball BA (1983) Carbon dioxide and agricultural yield: an assemblage and analysis of 430 prior observations. *Agronomy Journal*, **75**, 779–788.
- Körner C, Miglietta F (1994) Long term effects of naturally elevated CO₂ on Mediterranean grassland and forest trees. *Oecologia*, **99**, 343–351.
- Lashof DA (1989) The dynamic greenhouse feedback processes that may influence future concentrations of atmospheric trace gases and climatic change. *Climatic Change*, **14**, 235–242.
- van Loo EN, Schapendonk AHCM, De Vos ALF (1992) Effects of nitrogen supply on tillering dynamics and regrowth of perennial ryegrass populations. *Netherlands Journal of Agricultural Science*, **40**, 381–400.
- Long SP (1991) Modification of the response of photosynthetic productivity to rising CO₂ concentrations. Has its importance been underestimated? *Plant, Cell and Environment*, **14**, 729–739.
- Lutz JL, Gifford RM (1995) Carbon storage and productivity of a carbon dioxide enriched nitrogen limited grass sward after one years growth. *Journal of Biogeography*, **22**, 227–233.
- Morison JIL (1993) Responses of plants to CO₂ under limited conditions. *Vegetatio*, **104**, 193–209.
- Nijs I, Impens I, Van Hecke P (1992) Diurnal changes in the response of canopy photosynthetic rate to elevated CO₂ in a coupled temperature-light environment. *Photosynthesis Research*, **32**, 121–130.
- Owensby CE, Ham JM, Knapp AK, Bremer D, Auen LM (1997) Water vapor fluxes and their impact under elevated CO₂ in a C4-Tallgrass Prairie. *Global Change Biology*, **3**, 189–195.
- Parton WJ, Scurlock Ojima JMO, Schimel DS, Hall DS, SCOPEGRAM, group members DO (1995) Impact of climate change on grassland production and soil carbon world-wide. *Global Change Biology*, **1**, 13–22.
- Ryle GJA, Powell CE, Tewson V (1992) Effect of elevated CO₂ on the photosynthesis, respiration and growth of Perennial ryegrass. *Journal of Experimental Botany*, **43**, 811–818.
- Santrucek J, Sage RF (1996) Acclimation of stomatal conductance to a CO₂-enriched atmosphere and elevated temperature in *Chenopodium album*. *Australian Journal of Plant Physiology*, **23**, 467–478.
- Schapendonk AHCM, Goudriaan J (1996) The effect of increased CO₂ on the annual carbon balance of a *Lolium perenne* sward. In: *Photosynthesis: from Light to Biosphere* (ed. Mathis P) Vol. 5, pp. 779–784 Kluwer, The Netherlands.
- Schapendonk AHCM, Spitters CJT, Groot PJ (1989) Effects of water stress on photosynthesis and chlorophyll fluorescence of five potato cultivars. *Potato Research*, **32**, 17–32.
- Schapendonk AHCM, Spitters CJT, De Vos ALF (1990) Comparison of nitrogen utilisation of diploid and tetraploid perennial ryegrass genotypes using a hydroponic system. In: *Genetic Aspects of Mineral Nutrition* (ed. El Bassam), pp. 299–306.
- Schapendonk AHCM, Dijkstra P, Groenwold J, Pot CS, Van de Geijn SC (1996) Implications of elevated carbon dioxide concentration on *Lolium perenne* L. swards. Growth analyses and carbon balance. *Aspects of Applied Biology*, **45**, 31–40.
- Stitt M (1991) Rising CO₂ levels and their potential significance for carbon flow in photosynthetic cells. *Plant, Cell and Environment*, **14**, 741–762.
- Van de Geijn SC, Vos J, Groenwold J, Goudriaan J, Leffelaar PA (1994) The Wageningen Rhizolab- a facility to study soil-root-shoot-atmosphere interactions in crops. I. Description of main functions. *Plant and Soil*, **161**, 275–287.
- Van Ginkel JH, Gorissen A, Van Veen JA (1997) Carbon and nitrogen allocation in *Lolium perenne* in response to elevated atmospheric CO₂ with emphasis on soil carbon dynamics. *Plant and Soil*, **188-2**, in press.
- Wolfenden J, Diggle PJ (1995) Canopy gas exchange and growth of upland pasture swards in elevated CO₂. *New Phytologist*, **130**, 369–380.
- Wong SC, Cowan IR, Farquhar GD (1979) Stomatal conductance correlates with photosynthetic capacity. *Nature*, **282**, 424–426.
- Wong SC, Cowan IR, Farquhar GD (1985) Leaf conductance in relation to rate of CO₂ assimilation. III Influences of water stress and photoinhibition. *Plant Physiology*, **78**, 830–834.
- Zak DR, Pregitzer KS, Curtis PS, Teeri JA, Fogel RF, Radlett DL (1993) Elevated atmospheric CO₂ and feedback between carbon and nitrogen cycles. *Plant and Soil*, **151**, 105–117.