

Long-term effects of CO₂ enrichment and temperature increase on a temperate grass sward

I. Productivity and water use

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Abstract

Perennial ryegrass swards were grown in large containers on a soil, at two N fertilizer supplies, and were exposed over two years in highly ventilated plastic tunnels to elevated (700 $\mu\text{L L}^{-1}$ [CO₂]) or ambient atmospheric CO₂ concentration at outdoor temperature and to a 3 °C increase in air temperature in elevated CO₂. These swards were either fully irrigated (kept at field capacity) in each climatic condition (W+), or received the same amount of water in the three climate treatments (W-). In the latter case, the irrigation was adjusted to obtain a soil water deficit during summer and drainage in winter. Using a lysimeter approach, the evapotranspiration, the soil water balance, the productivity (dry-matter yield) and the water use efficiency of the grass swards were measured. During both years, elevated CO₂ increased the annual above-ground dry-matter yield of the W- swards, by 19% at N- and by 14% at N+. Elevated CO₂ modified yield to a variable extent during the growing season: a small, and sometime not significant effect (+6%, on average) was obtained in spring and in autumn, while the summer growth response was stronger (+48%, on average). In elevated CO₂, the temperature increase effect on the annual above-ground dry-matter yield was not significant, due to a gain in dry-matter yield in spring and in autumn which was compensated for by a lower summer productivity. Elevated CO₂ slightly reduced the evapotranspiration during the growing season and increased drainage by 9% during winter. A supplemental 3 °C in elevated CO₂ reduced the drainage by 29-34%, whereas the evapotranspiration was increased by 8 and 63% during the growing season and in winter, respectively. During the growing season, the soil moisture content at W- and at the high N supply declined gradually in the control climate, down to 20-30% of the water holding capacity at the last cut (September) before rewatering. This decline was partly alleviated under elevated CO₂ in 1993, but not in 1994, and was enhanced at +3 °C in elevated CO₂. The water use efficiency of the grass sward increased in elevated CO₂, on average, by 17 to 30% with no significant interaction with N supply or with the soil water deficit. The temperature increase effect on the annual mean of the water use efficiency was not significant. Highly significant multiple regression models show that elevated CO₂ effect on the dry-matter yield increased with air temperatures above 14.5 °C and was promoted by a larger soil moisture in elevated compared to ambient CO₂. The rate of change in relative dry-matter yield at +3 °C in elevated CO₂ became negative for air temperatures above 18.5 °C and was reduced by a lower soil moisture at the increased air temperature. Therefore, the altered climatic conditions acted both directly on the productivity and on the water use of the grass swards and, indirectly, through changes in the soil moisture content.

Abbreviations: 350 – outdoor climate; 700 – outdoor climate + 350 $\mu\text{L L}^{-1}$ [CO₂]; 700+ – outdoor climate + 350 $\mu\text{L L}^{-1}$ [CO₂] and + 3 °C; N- – low N fertilizer supply; N+ – high N fertilizer supply; DM – dry-matter; ET – evapotranspiration; ETM – maximal evapotranspiration; WUE – water use efficiency; W- – summer water limited swards; W+ – fully irrigated swards.

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Introduction

As shown by direct measurements (Keeling et al., 1995), the atmospheric CO₂ concentration is currently rising and it is expected that it will rise from 354 $\mu\text{L L}^{-1}$ [CO₂] in 1990 to 530 $\mu\text{L L}^{-1}$ [CO₂] in 2050, and could exceed 700 $\mu\text{L L}^{-1}$ [CO₂] in 2100 (Watson et al., 1990). This increase in atmospheric CO₂, with parallel increases in other 'greenhouse gases', is predicted to produce an increase in mean global temperatures of ca. 4 °C by 2100 (Watson et al., 1990), although recent models show that this temperature increase might be smaller (0.2 °C per decade) due to the cooling action of sulphate aerosols (Mitchell et al., 1995).

With perennial ryegrass, the impacts of elevated CO₂ have been assessed with spaced plants or simulated swards grown in a controlled environment (e.g. Ryle et al., 1992; Schenck et al., 1995) or with pot grown plants (Nijs et al., 1988, 1989), which may not have a sufficient rooting volume (Arp, 1991). However, major limitations of productivity through shortages of e.g. nitrogen and water have not yet been investigated year round under elevated CO₂ with this common temperate grass species.

Productivity in most temperate perennial ecosystems is usually limited by moisture and/or nutrient availability. Thus changes in water use efficiency or more efficient use of other nutrients would enhance ecosystem productivity. In particular, in a vegetation that is exposed frequently to water stress, the major response to elevated CO₂ is likely to be increased productivity from increased water use efficiency (Ham et al., 1995; Owensby et al., 1993). Increasing CO₂ concentration has been shown to cause partial stomatal closure (Morison, 1987). This decrease in stomatal conductance reduces transpiration per unit of leaf area, as has been observed many times (Kimball and Idso, 1983). Such a decrease in the rate of leaf water loss suggests the possibility of reductions in evapotranspiration (ET), and plant water requirements in elevated CO₂ (Idso and Brazel, 1985).

On the other hand, as the elevated CO₂ causes partial stomatal closure, the resultant decrease in transpirational cooling increases the foliage temperature (Idso et al., 1987; Morison and Gifford, 1984) and the leaf transpiration, thereby counteracting the effect of the CO₂ induced stomatal closure. Moreover, an increase in the total plant leaf area under elevated CO₂ may offset any decrease in ET per unit leaf area. Furthermore, a rise in air temperature could lead to an increase in the

evaporative demand and to larger water losses through evapotranspiration.

There have been only a few attempts to measure the effects of elevated CO₂ and temperature increase on ET or water use per unit land area of C₃ crops, as reviewed by Allen (1990), but generally the effects on ET have been small (Allen, 1990; Kimball et al., 1994). The longer-term, larger-scale measurements of water use efficiency may provide a more realistic response of the growth and water use of the vegetation, as highlighted by the reviews of Eamus (1991) and Morison (1993).

In the present study, perennial ryegrass swards (0.5 m²) were grown in field-like conditions, on a soil, at two nitrogen supplies and were subjected, or not, to elevated CO₂ and to temperature increase during two years. In order to mimic the average rainfall pattern in our site, all swards (except some fully irrigated controls) were supplied with the same amount of water. Using a lysimeter approach, ET, soil water balance, the WUE and the productivity of the grass swards were measured. We test the hypothesis that changes in the soil water status, resulting from altered water use, will interact with the direct effects of elevated CO₂ and temperature increase on the productivity of perennial grass swards. For example, we might expect that a lower water use in elevated CO₂ during spring will lead to an increase in water availability and in sward productivity during summer. On the other and, an increase in air temperature might deplete the soil water early in spring, thereby reinforcing the effects of the summer water deficit on sward growth.

Materials and methods

Climate control facility

A facility consisting of three 70 m² plastic tunnels covered with a polyethylene (120 μm) film, with outdoor temperature and CO₂ concentration tracking devices was used for the experiment. The climate inside each tunnel was controlled to obtain the following conditions:

- outdoor climate (350),
- elevated CO₂ (ambient + 350 $\mu\text{L L}^{-1}$ [CO₂]) (700),
- elevated CO₂ and temperature increase (ambient + 350 $\mu\text{L L}^{-1}$ [CO₂] and + 3 °C (700+)).

Within each tunnel, the air temperature was regulated, using an earth-tube heat exchanger (Falcimagne et al., 1995), consisting of 60, 15 m long, plastic pipes (0.13 m diameter) buried in the soil at depths ranging

between 0.6 and 2 m. During the day, the indoor air was circulated through this heat exchanger at a controlled rate (from 0 up to $11,000 \text{ m}^3 \text{ h}^{-1}$), or was returned to the tunnel through a by-pass. At night, part of the heat stored in the heat exchanger during day-time was released to the atmosphere, by circulating outdoor air. However, when the weather conditions changed too rapidly, resulting in a sharp drop in air temperature, the earth tube heat exchanger was no longer effective. In this case, a compressor (3 kW electrical power, Maneurop, France) was used to cool the air in the 350 and 700 tunnels. Additionally, at night, this compressor cooled the outdoor air which was passed through the earth tube heat exchanger.

The outdoor air, sampled at a height of 3 m, was circulated at a controlled rate (from 0 up to $7,000 \text{ m}^3 \text{ h}^{-1}$) in each tunnel. This air flow was enriched with pure CO_2 (L'Air Liquide, France) using a mass-flow controller (Tylan, USA) and, in the case of the 700+ tunnel, was heated by an electrical heater (6 kW). Inside each tunnel, the air was evenly distributed by 4 perforated plastic plena (0.45 m diameter), two at the height of the plant canopy, two located beneath the containers.

Air samples were taken every 15 minutes using a gas sampler (Siemens, Germany) and both the outdoor and the indoor CO_2 concentrations were measured with an IRGA (Mahiak, Germany). Analog-digital boards (Analog Devices, USA), as well as the data acquisition process, were controlled through a programme written in Pascal.

Micro-climate

The mean fraction of solar photosynthetically active radiation transmitted into the tunnel varied between 70 and 84% (Table 1). The plastic film covering the tunnels was changed every 6 months to prevent a further decline in solar radiation transmission. Means per regrowth (Table 1) show that the average difference in air temperature between the 350 and 700 tunnels was always below $0.5 \text{ }^\circ\text{C}$, whereas the average difference in air temperature between the 700 and 700+ tunnels ranged between 2.9 and $3.4 \text{ }^\circ\text{C}$. The 15 minute averages of the temperature were within $\pm 10\%$ of the temperature setpoint over 84% of the time.

The means per regrowth of the vapour pressure deficit (VPD) were not significantly ($p > 0.95$) different in the control (350) and elevated CO_2 (700) tunnels (Table 1). However, the VPD was increased, on average by 34% ($p < 0.05$, Wilcoxon's paired rank test), in the combined elevated CO_2 and temperature increase

treatment (Table 1). Since the average relative humidity of the air was only 5% lower at 700+ than at 700 (data not shown), the increase in VPD at 700+ resulted mainly from the $3 \text{ }^\circ\text{C}$ temperature increase in this climatic treatment.

No significant ($p > 0.95$, Wilcoxon's paired rank test) difference in CO_2 concentration occurred between the outdoor and the control (350) tunnel, on the one hand, and between the 700 and 700+ tunnels, on the other. On average during all regrowths, the CO_2 enrichment reached 346 and $344 \mu\text{L L}^{-1} [\text{CO}_2]$, respectively, in the 700 and 700+ tunnels (Table 1) and the 15 minute averages of the CO_2 concentration were within $\pm 5\%$ of the concentration setpoint over 94% of the time.

Sward management

In September 1991, 87 swards consisting of perennial ryegrass (*Lolium perenne* L., cv Préférence) were sown in 0.5 m^2 (45 cm deep) containers, filled with a well homogenised loamy soil (42% sand, 42% loam, 15% clay, 1% organic matter, C:N = 10, pH 6.9), and were grown outdoor. In March 1993, 18 months after sowing, these swards were assigned randomly to the 3 highly ventilated plastic tunnels: 350, 700 and 700+. The swards were placed side by side in two blocks, each consisting of two rows, and were randomized in each tunnel. The above-ground dry-matter yield was not significantly (ANOVA, data not shown) affected by the location of the swards inside each tunnel and therefore the results from the two blocks were pooled. From March 1993 to March 1995, the swards were grown continuously under the 350, 700 and 700+ climatic conditions.

All swards were cut simultaneously at 4 cm height in May, June, July, September and October (Table 1). A first spring cut was also made on April 15th. However, in 1993 at 350 and at 700, as the height of the grass canopy was below the cutting height of 4 cm no plant material was harvested at this April cut. Finally, all swards were cut at the end of winter, in early March 1993 and 1994, to eliminate the dead material accumulated since the last autumn cut. Aphid populations were controlled after each cut, alternatively with deltamethrin and with ethyl-parathion.

To avoid any growth limitation, each year, P, K and S were supplied at non limiting rates ($100 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$, $450 \text{ kg K}_2\text{O ha}^{-1}$, 120 kg S ha^{-1}) and CaO was supplied at 400 kg ha^{-1} . Two rates of nitrogen fertilizer supply were compared: 160 (N-) and 530 (N+) $\text{kg N ha}^{-1} \text{ y}^{-1}$. Nitrogen fertilizer was supplied

Table 1. Mean microclimate conditions in the 350 (outdoor climate), 700 (elevated CO₂) and 700+ (elevated CO₂ and 3 °C temperature increase) treatments in 1993 and in 1994. Outdoor rainfall (in mm) and irrigation supply in mm and expressed as a percentage of the irrigation of the control (350) fully irrigated (W+) sward (see text). Outdoor (Out.) and transmitted (Trans.) PAR. The air temperature, the vapour pressure deficit and the CO₂ concentration were measured at canopy height. The climatic sensors were scanned every second and 15 minute averages were recorded. The CO₂ concentration was measured every 15 minutes. The regrowth n°1 started on April 15th. The microclimate results are means and standard deviations per regrowth of the daily average values

Regrowth n° and cutting date	Out. rainfall (mm)	Indoor		PAR (mol m ⁻² d ⁻¹)		Temperature (°C)				Vapour pressure deficit (hecto Pa)				CO ₂ concentration (μL L ⁻¹ [CO ₂])					
		irrigation (mm)	(%)	Out.	Trans.	Out.	350	700	700+	Out.	350	700	700+	Out.	350	700	700+		
1993 1	May 17	68	74	104	31.2 (11.0)	22.0 (8.0)	13.0 (1.8)	12.8 (1.6)	13.1 (1.8)	16.1 (1.9)	9.5 (1.6)	6.8 (3.0)	7.2 (2.8)	10.1 (3.2)	363 (10)	364 (20)	710 (22)	698 (17)	
	2	June 21	62	137	93	42.6 (13.0)	31.5 (9.3)	17.4 (3.0)	17.7 (2.5)	17.2 (2.5)	20.3 (2.4)	11.5 (2.4)	8.7 (3.2)	8.0 (3.5)	11.8 (3.9)	347 (15)	348 (20)	696 (27)	682 (8)
	3	July 19	120	93	73	40.2 (12.6)	29.4 (9.2)	18.5 (2.4)	18.1 (2.3)	17.6 (2.7)	20.5 (2.7)	16.3 (5.2)	11.4 (4.2)	11.2 (3.7)	14.9 (4.6)	357 (19)	357 (20)	693 (199)	688 (27)
	4	Sept. 13	51	152	45	44.7 (14.2)	30.8 (9.9)	18.7 (3.5)	18.5 (3.4)	18.4 (3.4)	21.5 (3.2)	12.1 (4.4)	10.4 (4.0)	10.7 (3.7)	14.1 (4.0)	342 (8)	341 (8)	669 (19)	677 (32)
	5	Oct. 25	147	172	230	18.8 (10.1)	13.2 (6.2)	12.9 (3.5)	12.4 (3.5)	12.5 (3.4)	15.4 (3.1)	5.6 (3.2)	4.7 (3.4)	5.0 (2.6)	7.3 (3.0)	356 (8)	356 (10)	705 (9)	705 (9)
1994 1	May 27	103	112	94	37.4 (12.0)	31.3 (8.5)	14.3 (2.1)	15.0 (2.1)	15.0 (2.0)	18.2 (2.2)	8.3 (2.5)	6.5 (2.2)	7.0 (1.8)	9.6 (2.4)	342 (9)	342 (9)	692 (9)	693 (9)	
	2	June 28	40	96	97	44.0 (16.1)	34.2 (11.6)	17.7 (3.5)	18.3 (3.3)	18.4 (3.3)	21.7 (3.1)	11.7 (4.0)	8.4 (3.5)	8.2 (3.0)	11.2 (3.6)	337 (8)	337 (8)	687 (9)	687 (8)
	3	July 28	63	122	70	50.5 (11.2)	37.4 (7.8)	23.0 (2.6)	23.9 (2.4)	23.6 (2.4)	27.0 (2.2)	16.8 (4.7)	13.5 (4.6)	11.8 (3.4)	15.7 (4.1)	342 (8)	341 (8)	692 (8)	692 (8)
	4	Sept. 14	93	126	56	39.7 (11.6)	27.8 (7.5)	20.5 (3.3)	21.0 (3.0)	21.2 (3.1)	24.5 (2.9)	13.1 (4.6)	9.6 (4.3)	9.2 (3.6)	12.9 (4.1)	336 (12)	336 (12)	685 (12)	685 (10)
	5	Oct. 24	159	210	230	23.1 (8.5)	16.2 (4.4)	13.6 (3.2)	14.5 (2.7)	14.1 (2.8)	17.3 (2.6)	6.9 (2.7)	4.5 (2.7)	4.2 (1.9)	6.0 (2.3)	342 (15)	342 (15)	692 (16)	692 (15)

as NH₄NO₃ after the May, June, July and September cuts at a rate of 30 (N-) or 100 (N+) kg N ha⁻¹. This dose was increased by 30% (39 and 130 kg N ha⁻¹ at N- and N+, respectively, applied in April) before the reproductive regrowth, which occurred in May. Finally, at 700+, due to the earlier spring growth, the swards were supplied in March with a supplemental 20 (N-) or 70 (N+) kg N ha⁻¹.

Above-ground dry-matter yield, leaf area and water use efficiency

At harvest, all swards were cut at 4 cm height with a battery-powered clipper and, as no guard rows were used, all the cut plant material was sampled. A sub-sample was taken, the leaf blade, pseudo-stem and dead material were separated and the leaf blade area was measured with an electronic planimeter (Li-Cor, L13100, USA). The sub-samples and the remainder of the harvested dry-matter were dried (80 °C, 48 h) and weighed. The leaf area index at harvest, standardised

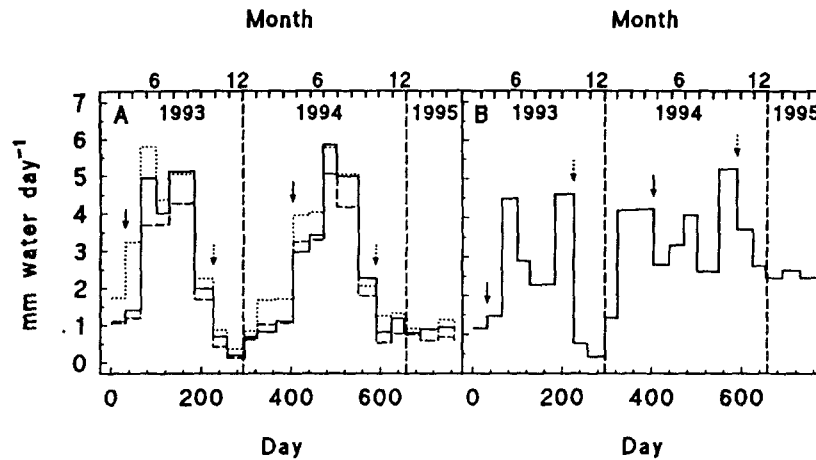


Figure 1. Irrigation supply (mm d^{-1}) to the fully irrigated (W+) swards (A) and to the summer water limited (W-) swards (B) in the three climatic treatments during the time course (in days, day 0 in March 1993) of the experiment. 350 (—), control at $350 \mu\text{L L}^{-1} [\text{CO}_2]$; 700 (---), elevated CO_2 ($+350 \mu\text{L L}^{-1} [\text{CO}_2]$); 700+ (.....), elevated CO_2 ($+350 \mu\text{L L}^{-1} [\text{CO}_2]$) and temperature increase ($+3^\circ\text{C}$). Vertical arrows denote the beginning (\downarrow) and the end (\uparrow) of the growing season (from April to October).

not to include the area of the sheath material, was calculated by multiplying the DM yield by the weight per unit leaf blade area of the subsample. The percentage of dead material in the harvests was always lower than 10 or 20% at N- and N+, respectively. The water use efficiency (WUE) was calculated as the ratio of the above-ground dry-matter yield to evapotranspiration during one regrowth.

Water supply

Water supply was adjusted to simulate the average changes in water availability that usually occur in our site: good water availability during spring; summer water deficit; autumn and winter drainage. All swards were irrigated twice weekly in summer (June to September) and once weekly during the rest of the year. The irrigation volumes were delivered to each container using a water-meter (precision: ± 0.1 litre).

In each tunnel, one N+ ryegrass sward was fully irrigated (W+) to keep a soil water content close to the field capacity throughout the year: each week (or twice weekly in summer), the containers were weighed (Dina, France; precision ± 0.2 kg) and then irrigated until the weight at the field capacity was reached (Fig. 1A). The ET of these fully irrigated swards differed from the outdoor potential ET (also estimated by weighing a fully irrigated sward, data not shown), due: i) to indoor-outdoor differences in wind velocity and radiative balance; ii) to elevated CO_2 or increased

temperature effects on the ET in the 700 and 700+ tunnels.

Apart from the three fully irrigated (W+) swards, the irrigation volumes delivered to all remaining (summer water limited, W-) swards were equal (Fig. 1B). These were calculated as follows:

- During the spring growing period, that is from mid-April to the June cut, the irrigation rate was equal to that supplied to the fully irrigated N+ sward in the control (350) tunnel (Table 1).
- During the July and September regrowths, the irrigation rate was equal, to 70 and 50%, respectively, of that supplied to the fully irrigated sward in the control (350) tunnel (Table 1).
- From mid-September to mid-April, drainage was obtained by supplying the same amount of water in excess to all swards (10 L per container, each week).

This procedure allowed to mimic the summer water deficit that occurs in our site, while ensuring that all W-swards in the three climatic conditions received the same amount of water throughout the year. A total of 628 and 666 mm water was supplied to the W-swards during the growing seasons in 1993 and 1994, respectively (Table 1, Fig. 1).

Water balance

The components of the soil and vegetation water balance were measured, using a lysimeter approach:

$$\text{Irrigation} = \text{ET} + \text{drainage} + \text{soil moisture change}$$

During winter, drainage water was collected and drainage amounts were weighed. The weight of freely drained containers at field capacity reached on average 380 kg. At the start and at the end of the experiment, the weight loss from the field capacity to the permanent wilting point, was measured with two containers. The soil water holding capacity was equal to the mean weight loss, which reached 42 ± 2 kg per container, that is 84 mm. The moisture content of the soil at the permanent wilting point was then measured, by sampling at random 10 replicate soil cores on two containers. The soil samples were weighed and oven dried at 110 °C during 48 h.

At each cut (or each month during winter), two replicate N-W- and N+W- swards in each tunnel were weighed to determine their soil water content and the amount of water lost by ET and/or (in winter) by drainage. Drainage amounts were measured by weighing before each irrigation. The soil water content was expressed as a percentage of the soil water holding capacity.

Soil water potential and (pre-dawn) leaf water potential

A pressure membrane apparatus was used to measure the matric potential of the soil at various water contents. These measurements were made, with replicates, on bare soil sampled before the start of the experiment and on soil sampled at the end of the experiment from the 700, N+ treatment. The field capacity was at $0.21 \text{ g H}_2\text{O g}^{-1}$ dry soil and the permanent wilting point (1.6 MPa) at, approximately, $0.04 \text{ g H}_2\text{O g}^{-1}$ dry soil.

At the end of the experiment, on a drying soil, at soil moisture contents varying between 100 and 0% of the water holding capacity, the pre-dawn (04:00 h solar time) leaf water potential was estimated as the xylem pressure potential with a pressure chamber. The most recently fully expanded leaf was excised with a sharp razor blade, enclosed in a previously humidified airtight plastic bag, which was stored for a few minutes at ambient temperature in the dark prior to measurement. Measurements were replicated on leaves excised from ten tillers selected at random.

Statistical analysis

The statistical design is a split-plot with the climate as main factor and N supply and water supply as split factors. A disadvantage is the absence of true replication (duplicates of the tunnels), which can be compensat-

ed for by the replication of swards within the tunnel and by careful scrutiny of the tunnels' microclimate (see above). At the start of the experiment, there were in each tunnel 14 replicate swards for the N- and for the N+ (W-) treatments. However, in order to study the below-ground compartment (see Soussana et al., 1996) some of the W- swards were sampled destructively. This resulted in a gradual decline in the number of replicates available at each regrowth (in 1993, 14, 13, 12, 11 and 11 replicates were available in May, June, July, September and October, respectively, and, in 1994, the same figures were equal to 6, 6, 5, 5 and 4). One replicate of the W+N+ treatment (control fully irrigated sward) was available throughout the experiment in each tunnel.

A repeated measure ANOVA was performed on the annual means of the DM yield and of the water use efficiency in the W- treatment, with the climate and the N supply as factors and the year (1993 or 1994) as the time variable. The seasonal means of the components of the water balance (proportions) were first transformed (by the arcsin of the square root) and then analysed in the same way by a repeated measure ANOVA. The normality of the data was checked using normal probability plots and their homogeneity of variance by applying the variance test ratio.

Stepwise multiple regressions were used to account for the effects of environmental variables (temperature, soil moisture, radiation, vapour pressure deficit of the air) on the above-ground DM yield. The independent variables selected in the final model had a F ratio larger than 4 and were all significant at ($p < 0.05$). All statistical analysis were made using the software Statgraphics Plus (Manugistics, USA).

Results

Above-ground dry matter yield

During both years, elevated CO₂ (700) increased the annual above-ground DM yield of the W- swards by 1 and 2 t DM ha⁻¹ y⁻¹ at N- and N+, respectively, that is by +19 and +14% (Table 2A). This increase in DM yield was highly significant (Table 2B). Elevated CO₂ modified yield to a variable extent during the growing season: a small, and sometimes not significant effect, was obtained in spring and autumn, while the summer response was stronger and was always significant (Fig. 2). The mean percentage increase in DM yield was +48% for the July and September cuts and +6% for the

Table 2. Mean (\pm s.e.) annual above-ground dry-matter yield of perennial ryegrass swards ($t DM ha^{-1}$) (A) and ANOVA (B) for the mean annual dry-matter yield of the summer water limited swards. Water supply: W-, summer water limited (see Materials and methods) sward; W+, fully irrigated control sward. N supply: N-, $160 kg N ha^{-1} y^{-1}$; N+, $530 kg N ha^{-1} y^{-1}$. 350, control climate; 700, $+350 \mu L L^{-1} [CO_2]$; 700+, $+350 \mu L L^{-1} [CO_2]$ and $+3^\circ C$. In B), the ANOVA was performed with the climate (350, 700 or 700+), the N supply and the year as factors and their interactions. (*, **, ***) denote a significant difference in the ANOVA at $p < 0.05$, $p < 0.01$, $p < 0.001$, respectively. Planned contrasts between 700 and 350 and 700+ and 700, respectively, were tested according to the L.S.D. method, to reveal whether the CO_2 and temperature effects were significant

A	W-				W+	
	N-		N+		N+	
	1993	1994	1993	1994	1993	1994
350	4.8 \pm 0.2	5.6 \pm 0.1	14.2 \pm 0.6	15.7 \pm 0.3	14.7	16.2
700	5.7 \pm 0.2	6.6 \pm 0.3	16.2 \pm 0.5	17.9 \pm 0.6	18.7	17.9
700+	5.8 \pm 0.4	6.5 \pm 0.4	14.4 \pm 0.4	18.4 \pm 0.3	19.8	19.4

B	CO_2	Temp.	N	Year	CO_2 $\times N$	Temp. $\times N$	CO_2 \times Year	Temp. \times Year	N \times Year
Statistical significance	***	N.S.	***	***	N.S.	N.S.	N.S.	N.S.	**

Table 3. Seasonal mean (\pm s.e.) of the components of the mean soil water balance of the summer water limited (W-) perennial ryegrass swards, expressed as a percentage of the irrigation supply (A) and repeated measure ANOVA for the seasonal means of the components of the water balance (B). The components of the soil water balance were calculated during the growing season (April through October) and during the winter of 1993 and 1994. ET/I, mean evapotranspiration to irrigation ratio (%); D/I, mean evapotranspiration to irrigation ratio (%). Same abbreviations as in Table 2. Please note that the total of evapotranspiration and drainage does not account for 100% of the irrigation supply, due to small changes in the soil moisture content from the beginning to the end of the period considered. The results in A are the mean of two replicate containers. Planned contrasts between 700 and 350 and 700+ and 700, respectively, were tested according to the L.S.D. method, to reveal whether the CO_2 and temperature effects were significant. In B, for conciseness, the interactions between factors were omitted as they were not significant

A	Growing season				Winter				
	N-		N+		N-		N+		
	1993	1994	1993	1994	1993	1994	1993	1994	
ET/I (%)	350	81 \pm 1	78 \pm 1	89 \pm 1	85 \pm 1	31 \pm 4	18 \pm 2	50 \pm 3	31 \pm 1
	700	80 \pm 1	76 \pm 2	86 \pm 2	84 \pm 1	31 \pm 5	18 \pm 1	40 \pm 5	26 \pm 2
	700+	84 \pm 1	83 \pm 1	93 \pm 1	92 \pm 2	47 \pm 6	31 \pm 2	69 \pm 4	41 \pm 2
D/I (%)	350	17 \pm 3	21 \pm 1	10 \pm 1	16 \pm 1	63 \pm 4	88 \pm 1	43 \pm 5	71 \pm 2
	700	17 \pm 1	23 \pm 2	11 \pm 1	17 \pm 1	66 \pm 5	90 \pm 2	54 \pm 5	80 \pm 3
	700+	13 \pm 2	17 \pm 1	7 \pm 2	8 \pm 2	50 \pm 4	72 \pm 2	25 \pm 3	60 \pm 2

B	ET/I		D/I	
	Growing season	Winter	Growing season	Winter
CO_2	*	N.S.	N.S.	*
Temperature	***	***	***	***
N	***	***	***	***
Year	***	***	***	***

May, June and October cuts (means of two years and of two N treatments) (Fig. 2).

A $3^\circ C$ increase in air temperature at elevated CO_2 had a negative effect on the mean annual DM yield in

1993 at N+, but this was not observed in 1994 (Table 2A) and the effect of a supplemental $3^\circ C$ on the annual DM yield was not significant (Table 2B). The temperature increase allowed earlier sward growth in spring

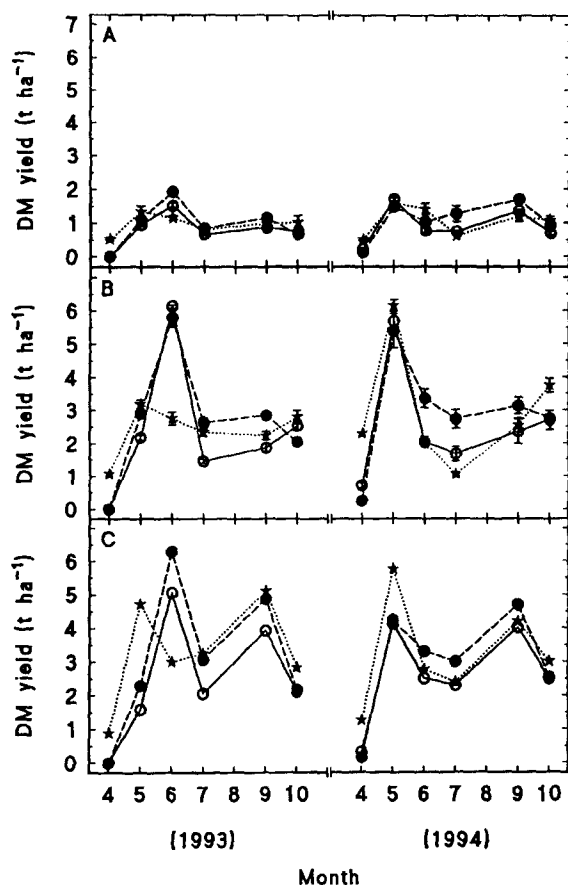


Figure 2. Above-ground dry-matter yield (t ha^{-1}) of perennial ryegrass swards at cuts in 1993 and in 1994. (A) N- W-, low inorganic N supply ($160 \text{ kg N ha}^{-1} \text{ y}^{-1}$); (B) N+ W-, high inorganic N supply ($530 \text{ kg N ha}^{-1} \text{ y}^{-1}$). (C) fully irrigated control N+ W+ swards. 350 (○—), control at $350 \mu\text{L L}^{-1} [\text{CO}_2]$; 700 (●—), elevated CO_2 ($+350 \mu\text{L L}^{-1} [\text{CO}_2]$); 700+ (*—), elevated CO_2 ($+350 \mu\text{L L}^{-1} [\text{CO}_2]$) and temperature increase ($+3 \text{ }^\circ\text{C}$). The vertical bars denote the standard error of the mean whenever it was larger than the symbol size.

(April cut) and increased DM production at the October cut (Fig. 2). In 1993, the comparison between ambient temperature and $+3 \text{ }^\circ\text{C}$ (700 and 700+, Fig. 2) is rendered more difficult at the May cut by the fact that the 700 swards had not been cut previously in April, due to a lack of dry-matter accumulation above 4 cm. During the June, July and September cuts, the sward DM yield was mostly reduced by the $3 \text{ }^\circ\text{C}$ temperature increase in elevated CO_2 (Fig. 2). No significant interaction occurred between the effects of elevated CO_2 and increased temperature, on the one hand, and of N supply or year, on the other (Table 2B).

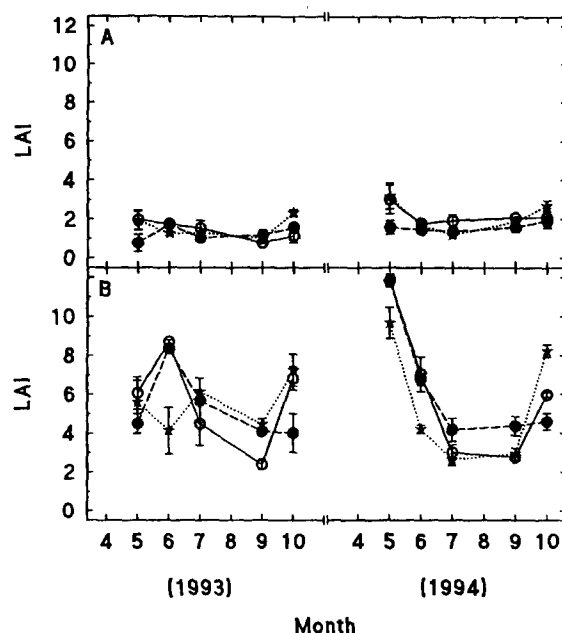


Figure 3. Leaf area index of perennial ryegrass swards at successive cuts from May to October 1993 and 1994. (A) N-, low inorganic N supply ($160 \text{ kg N ha}^{-1} \text{ y}^{-1}$); (B) N+, high inorganic N supply ($530 \text{ kg N ha}^{-1} \text{ y}^{-1}$). 350 (○—), control at $350 \mu\text{L L}^{-1} [\text{CO}_2]$; 700 (●—), elevated CO_2 ($+350 \mu\text{L L}^{-1} [\text{CO}_2]$); 700+ (*—), elevated CO_2 ($+350 \mu\text{L L}^{-1} [\text{CO}_2]$) and temperature increase ($+3 \text{ }^\circ\text{C}$). The results are the mean of two replicates. The vertical bars denote the standard error of the mean whenever it was larger than the symbol size.

Leaf area index

With the summer water limited (W-) swards, at N- the leaf area index (LAI) was below 3 at all cuts (Fig. 3A), indicating that solar radiation was not fully intercepted by the grass canopy and that a significant soil evaporation occurred. At N+, higher LAI values (ranging between 4 and 12) were recorded during spring and autumn (May, June and October cuts).

Elevated CO_2 lowered the sward LAI at N-W- in May (Fig. 3A). At N+W-, elevated CO_2 increased significantly the LAI at the July and September cuts (Fig. 3B), when water was limiting (see below). On the contrary, at the October cut, CO_2 enrichment resulted in lower values of the grass LAI (Fig. 3). A supplemental $3 \text{ }^\circ\text{C}$ in elevated CO_2 increased the grass sward LAI in May and in October (except for May 1994) (Fig. 3), that is for cool air temperatures (Table 1). For the summer regrowths (June, July, September) an increased air temperature had no effect at N-, but depressed significantly the LAI at N+ in 1994 and in June 1993 (Fig. 3B).

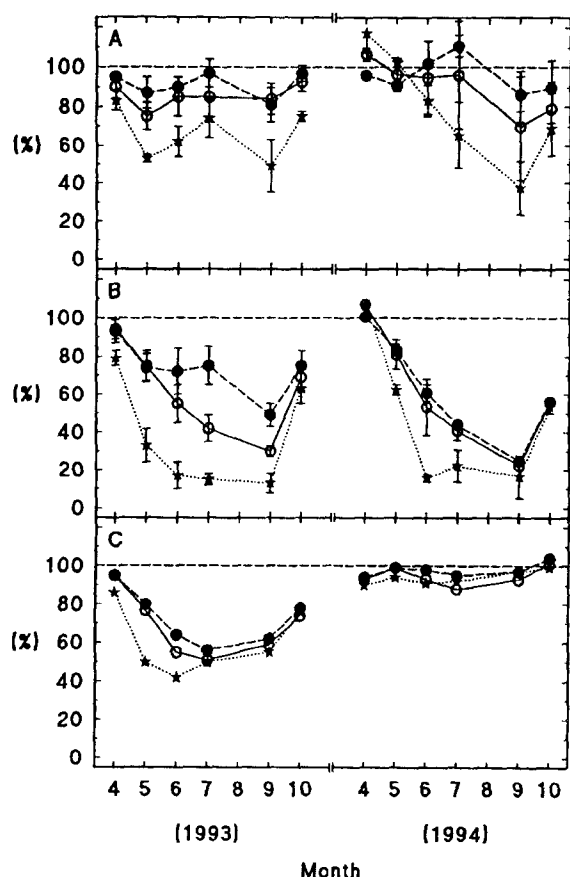


Figure 4. Mean soil moisture content during regrowth, expressed as a percentage of the soil water holding capacity (see Materials and methods) during the growing seasons in 1993 and in 1994. (A) N-W-, low inorganic N supply ($160 \text{ kg N ha}^{-1} \text{ y}^{-1}$); (B) N+ W-, high inorganic N supply ($530 \text{ kg N ha}^{-1} \text{ y}^{-1}$). (C) fully irrigated control (N+ W+) swards. 350 (○—), control at $350 \mu\text{L L}^{-1} [\text{CO}_2]$; 700 (●—), elevated CO_2 ($+350 \mu\text{L L}^{-1} [\text{CO}_2]$); 700+ (×···), elevated CO_2 ($+350 \mu\text{L L}^{-1} [\text{CO}_2]$) and temperature increase ($+3 \text{ }^\circ\text{C}$). The results at W- are the mean of two replicate containers. The vertical bars denote the standard error of the mean whenever it was larger than the symbol size. For the control fully irrigated swards, please note that only one container was used.

Soil moisture content

With the (350) N+ summer water limited (W-) swards, the soil moisture content declined gradually down to 20-30% of the water holding capacity at the last cut (September) before rewatering (Fig. 4B). This decline was partly alleviated under elevated CO_2 in 1993, but not in 1994 (Fig. 4B). At N-, the sward ET was restricted (Table 3), due to a lower LAI (Fig. 3), which led to larger soil moisture contents, especially in elevated CO_2 (Fig. 4A).

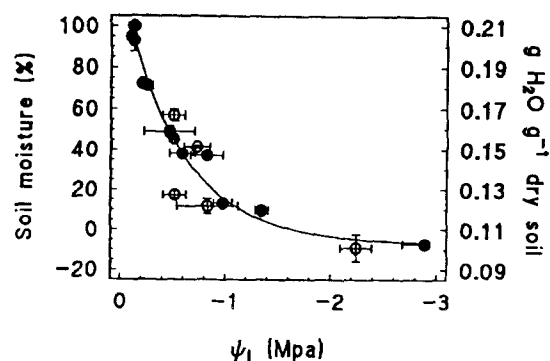


Figure 5. Leaf base water potential (Ψ_1 , MPa) of perennial ryegrass as a function of the soil moisture content. The soil moisture content was expressed either as a percentage of the water holding capacity (left axis), or in g water g^{-1} dry soil (right axis). Open symbols are for the low inorganic N supply (N-); closed symbols are for the high inorganic N supply (N+). The results from the 3 different climatic treatments were confounded as they did not differ at ($p < 0.05$) and are the mean of 30 replicate leaves. The horizontal and vertical bars denote the standard error of the mean whenever it was larger than the symbol size.

A $3 \text{ }^\circ\text{C}$ temperature increase in elevated CO_2 resulted in a fast decline in the soil moisture content of the W- swards. At N+, a minimum value (at approximately 15% of the water holding capacity) was reached in June and the soil moisture content remained close to this minimum until September. At N-, the temperature increase also resulted in a significant depletion of the soil water, but higher minimum values (at 40-50% of the water holding capacity of the soil) were obtained (Fig. 4).

With the three W+ swards, during the first year of the experiment, as the irrigation volume supplied during one week was calculated to compensate exactly the losses of the preceding week and as the evaporative demand increased gradually during the growing season, the irrigation supply lagged behind the ET, which resulted in declining soil moisture contents (Fig. 4C). This problem was solved in 1994, by supplying 5 L water in advance at each irrigation to each container. Thereby, in 1994, the soil moisture content was kept close to the field capacity in the three W+ swards (Fig. 4C).

Pre-dawn leaf water potential

For a given soil moisture, no significant difference in pre-dawn leaf water potential occurred between the climate treatments or between the N- and N+ swards (Fig. 5). When the soil moisture content was above 50% of the water holding capacity, the pre-dawn leaf

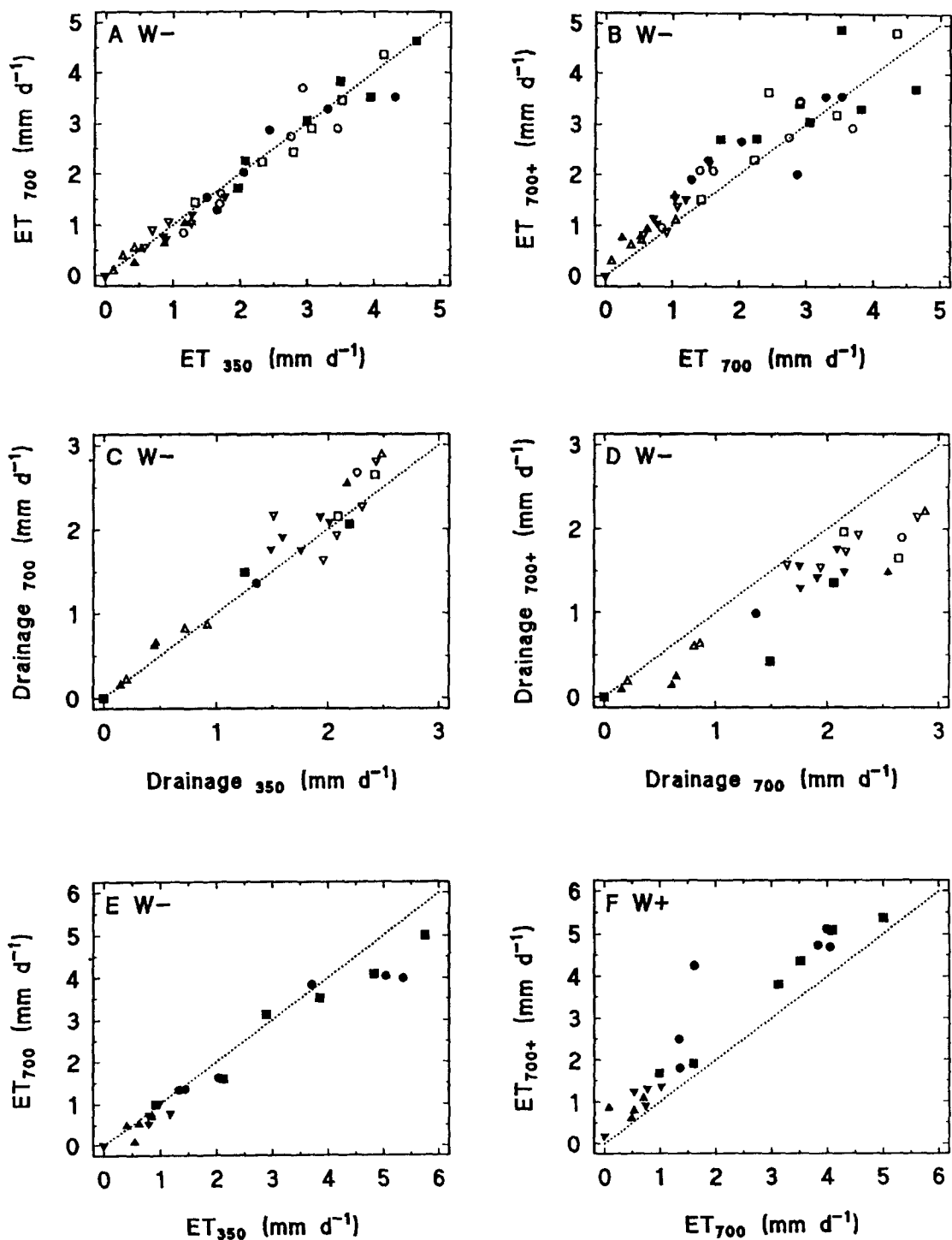


Figure 6. Elevated CO₂ and temperature increase effects on (A,B) the evapotranspiration (ET) and (C,D) the drainage of summer water limited (W-) perennial ryegrass swards and on the ET of the fully irrigated (W+) perennial ryegrass swards. Open symbols are for low inorganic N supply (N-, 160 kg N ha⁻¹ y⁻¹), solid symbols are for high inorganic N supply (N+, 530 kg N ha⁻¹ y⁻¹). (●, ○), growing season in 1993; (■, □), growing season in 1994; (▲, △), winter period in 1993; (▼, ▽) winter period in 1994. The results are the mean of two replicate containers. The dotted line is the 1:1 line.

water potential decreased little and was not lower than -0.6 MPa (Fig. 5). For lower soil moisture contents, the decline in the pre-dawn leaf water potential was greater and values in the range of -0.8 to -1.4 MPa were reached for 20% of the soil water holding capacity (Fig. 5).

Water balance

With the N- and N+ summer water limited (W-) swards, the ET and the drainage were apparently similar in elevated (700) and in ambient CO_2 (Fig. 6A and C). An increase in air temperature reduced the drainage of the W- swards and, for low (below 2 mm d^{-1}) values of ET in the control (700), increased the evapotranspiration (Fig. 6B, D). With the control W+ swards, drainage was avoided and the soil moisture changes were restricted, these swards being kept close to the field capacity. In this case, elevated CO_2 reduced, on average, the ET by 15% whereas a supplemental 3°C in elevated CO_2 increased it by 37% (Fig. 6E and F).

The average ET and drainage fluxes at W- are listed in Table 3, during the growing season (mid April to mid October) and during winter, as a percentage of the irrigation supply. With the W- swards, increasing N supply reduced significantly the average drainage and increased the average ET both during the growing season and in winter (Table 3). Averaged over two years, elevated CO_2 reduced significantly the ET during the growing season (by 2% on average) and increased significantly the drainage during winter (by 9% on average, Table 3). Over two years, a supplemental 3°C in elevated CO_2 led to a highly significant increase in the ET of the W-swards (by +8 and +63% during the growing season and in winter, respectively), while the average drainage was significantly reduced (by 34 and 29% during the growing season and in winter, respectively) (Table 3).

Actual to potential evapotranspiration and timing of the water deficit

As water deficit restricts transpiration, the ratio of the actual to the potential transpiration is usually a good index of the occurrence of water deficit. In 1994, as the W+ swards were kept at the field capacity, their ET was very close to the potential ET (ETM) in the sense of Penman (1956). Nevertheless, at the field capacity, both the ET (Fig. 7A) and the DM yield (Fig. 7B) of the W- swards were greater than that of the W+ swards.

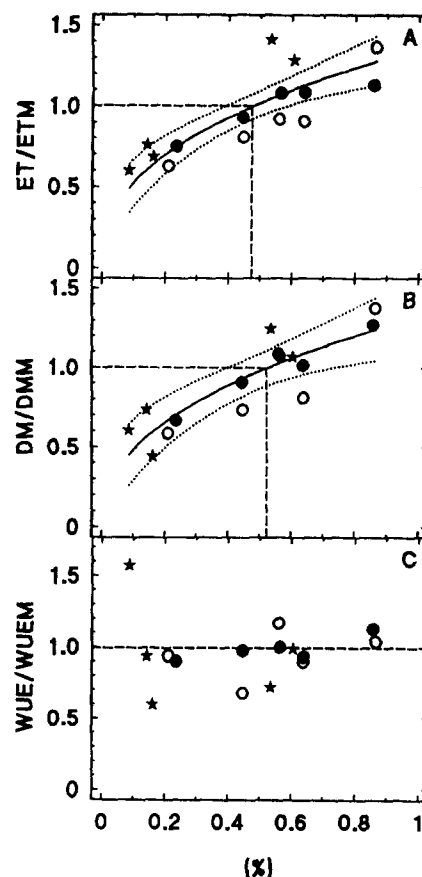


Figure 7. Actual to potential evapotranspiration ratio (A), actual to 'potential' dry-matter yield (B) and actual to 'potential' WUE (C) of the N+ swards in 1994, as a function of the soil moisture content expressed as a percentage of the water holding capacity. The potential evapotranspiration (ETM), dry-matter yield (DMM) and water use efficiency (WUEM) are for the fully irrigated swards (N+ W+), which were maintained at the field capacity in 1994. The soil moisture content at W- was expressed as a percentage of the water holding capacity of the soil. The results are the mean of 2 replicate containers. 350 (○), control at $350 \mu\text{L L}^{-1} [\text{CO}_2]$; 700 (●), elevated CO_2 ($+350 \mu\text{L L}^{-1} [\text{CO}_2]$); 700+ (*), elevated CO_2 ($+350 \mu\text{L L}^{-1} [\text{CO}_2]$) and temperature increase ($+3^\circ\text{C}$). The results were fitted to the following highly significant ($p < 0.001$) power models: (A) $Y = (1.36 \pm 0.07) X^{(0.42 \pm 0.06)}$; (B) $Y = (1.31 \pm 0.07) X^{(0.43 \pm 0.07)}$. The dotted lines depict the confidence interval at ($p < 0.05$) of the corresponding regression.

The ET to ETM ratio declined with declining soil moisture contents but, compared to the ETM, a restriction in ET was observed only below 50% of the soil water holding capacity (Fig. 7A). From these results, it can be argued that the grass swards were not water limited when the soil moisture content was above 50% of the water holding capacity, as in this case no reduction in ET or in DM yield occurred (Fig. 7) and as

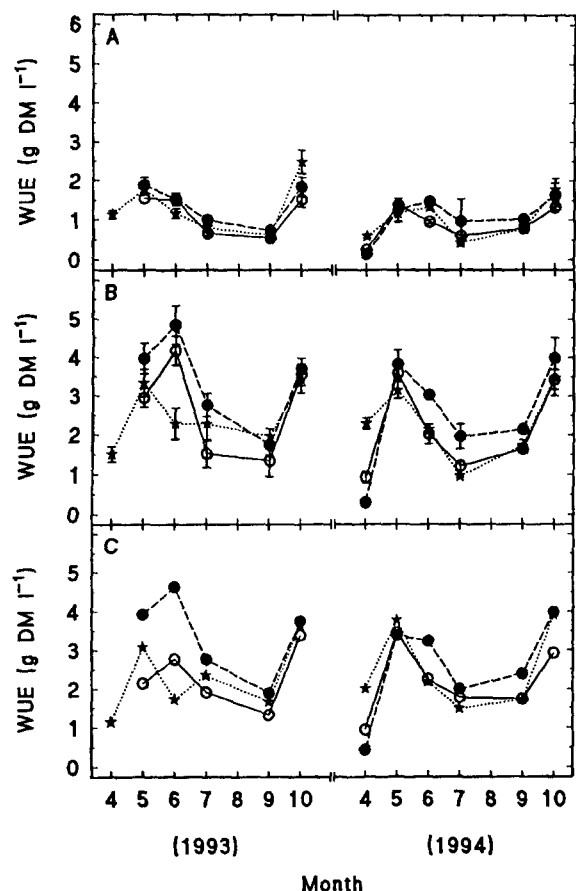


Figure 8. Water use efficiency of perennial ryegrass swards during 1993 and 1994. (A) N-W-, low inorganic N supply ($160 \text{ kg N ha}^{-1} \text{ y}^{-1}$); (B) N+ W-, high inorganic N supply ($530 \text{ kg N ha}^{-1} \text{ y}^{-1}$). (C) fully irrigated control (N+ W+) swards. 350 (\circ —), control at $350 \mu\text{L L}^{-1} [\text{CO}_2]$; 700 (\bullet -), elevated CO_2 ($+350 \mu\text{L L}^{-1} [\text{CO}_2]$); 700+ ($\times \cdots$), elevated CO_2 ($+350 \mu\text{L L}^{-1} [\text{CO}_2]$) and temperature increase ($+3 \text{ }^\circ\text{C}$). The vertical bars denote the standard error of the mean whenever it was larger than the symbol size. For the control fully irrigated swards, please note that only one replicate container was used.

the pre-dawn leaf water potential was above -0.6 MPa (Fig. 5).

Accordingly, except for the 700+ treatment at the September 1994 cut, no water deficit occurred at N- (Fig. 4). Also, with the three W+ swards no water deficit occurred, apart from a possible mild deficit in June 1993 at 700+ (Fig. 4). Yet, at N+ with the summer water limited (W-) swards, water deficit occurred but was restricted to the July and September cuts at 350 and 700, whereas it began earlier, at the May or at the June cut, and was much stronger (soil moisture contents below 20% of the water holding capacity) at 700+.

Plant water deficits may be considerable in 'moist' soils under high evaporative demand or, conversely small under low evaporative demand even in a relatively 'dry' soil (Kramer, 1983). Therefore, we should not rule out the possibility that perennial ryegrass plants experienced transient water deficits when the evaporative demand was high, despite soil moisture contents larger than 50% of the water holding capacity.

Water use efficiency

The grass WUE displayed usually a seasonal minimum during summer and a peak in spring and autumn (Fig. 8). The summer decline of the grass WUE occurred both with and without a soil water deficit (Fig. 8), presumably as a result of the increased vapour pressure deficit of the air during summer (Table 1), since it was observed that both variables were negatively correlated ($p < 0.001$, data not shown). During the growing season, the WUE was significantly higher at N+ than at N- (Table 4), but was apparently little affected at N+ by full irrigation (Fig. 8). The elevated CO_2 effect on the annual mean of the WUE of the W- swards was significant ($p < 0.05$, Table 4) and ranged from +17 to +30% (Table 4).

At W-, a supplemental $3 \text{ }^\circ\text{C}$ in elevated CO_2 depressed significantly the WUE for some summer cuts (Fig. 8). Nevertheless, the temperature increase effect on WUE was positive at the start of spring (April cut) and there was no significant effect of a supplemental $3 \text{ }^\circ\text{C}$ on the annual mean of the WUE (Table 4). At N+, the WUE of the W- and W+ swards were similar during the summer cuts (Fig. 8 C). Moreover, in 1994, the soil water deficit experienced by the W- swards during summer did not cause large changes in their WUE, as compared to that of the control fully irrigated (W+) swards (Fig. 7C).

Discussion

Above-ground dry-matter yield in elevated CO_2

Elevated CO_2 resulted in a 10% increase in the dry-matter yield of perennial ryegrass spaced plants grown at an optimal ($20/15$) $^\circ\text{C}$ temperature and under optimal nutrients supply (Ryle et al., 1992). In the present study, with soil grown and summer water limited (W-) perennial ryegrass swards, the elevated CO_2 effects on the annual dry-matter yield was somewhat larger: +14 to +19%, in good agreement with results by Schenck

Table 4. Annual mean (\pm s.e.) of the grass water use efficiency (g DM L^{-1}) during the growing season (April through October cuts) (A) and repeated measure ANOVA for the mean annual water use efficiency (WUE) of the summer water limited (W-) swards (B). Same abbreviations as in Table 2. The results in A are the mean of two replicate containers at W- and of one replicate for the control swards at W+. Planned contrasts between 700 and 350 and 700+ and 700, respectively, were tested according to the L.S.D. method, to reveal whether the CO_2 and temperature effects were significant. In B, for conciseness, the interactions between factors were omitted as they were not significant

A	W-				W+	
	N-		N+		N+	
	1993	1994	1993	1994	1993	1994
350	0.97 \pm 0.2	0.89 \pm 0.1	2.3 \pm 0.4	2.1 \pm 0.3	1.9	2.2
700	1.2 \pm 0.2	1.1 \pm 0.2	2.8 \pm 0.5	2.5 \pm 0.4	2.8	2.6
700+	1.3 \pm 0.3	1.0 \pm 0.2	2.5 \pm 0.4	2.3 \pm 0.4	2.3	2.5
B		CO_2	Temp.	N	Year	
Statistical significance		**	N.S.	***	N. S.	

et al. (1995) for a controlled environment experiment at five different plant densities.

As the swards were cut on a given date and not at a given weight, the LAI was usually not the same in elevated and in ambient CO_2 (Fig. 3). Using a modelling approach, Nijs et al. (1993), have suggested that differences in LAI between ambient and elevated CO_2 treatments might modify light interception and shoot respiration and therefore interact with the effects of elevated CO_2 . Yet, the results from Schenck et al. (1995) indicate that the effect of CO_2 enrichment on the shoot dry-matter of perennial ryegrass is independent of plant density and thus of light and nitrogen limitation. In agreement with this report, we did not observe any significant interaction between the effects of elevated CO_2 and N supply on the annual above-ground dry-matter yield (Table 2B).

The elevated CO_2 effect on the W- swards varied strongly between cuts (Fig. 2). Indeed, several environmental factors (air temperature, vapour pressure deficit, daily radiation input, soil water deficit, Table 1), which were positively correlated throughout the growing season (data not shown), might have interacted with elevated CO_2 . Moreover, the timing of drought between CO_2 treatments was different in 1993, due to the time course of water depletion by the swards (Fig. 4). This makes it more difficult to ascertain the exact causes of the seasonality of the elevated CO_2 effect on the above-ground productivity of the N- and N+ summer water limited swards.

Among the climatic factors, the relative rate of change in the dry-matter yield in elevated CO_2 was most correlated with the air temperature and with the

vapour pressure deficit of the air ($r^2 = 0.61$, $p < 0.0001$ and $r^2 = 0.52$, $p < 0.001$, respectively) and, among the soil water factors, with the difference in soil water content between elevated and ambient CO_2 ($r^2 = 0.33$, $p < 0.005$), whereas the average soil moisture deficit displayed a much weaker correlation ($r^2 = 0.18$, $p < 0.05$). Indeed, despite a soil moisture content close to the field capacity, the yield response to elevated CO_2 of the W+ swards was apparently larger in summer than in spring or autumn (Fig. 2). However, this point can not be ascertained since only one replicate was available at W+.

Table 5 shows that the mean daily air temperature during regrowth and the difference in soil moisture between 700 and 350 accounted for 68% of the total variability of the relative rate of change in the DM yield in elevated CO_2 . From this regression, provided soil moistures at 350 and at 700 are similar, the relative rate of change in yield in elevated CO_2 is positive for a mean air temperature during regrowth above (14.5 ± 1) $^\circ\text{C}$. Also, for a given air temperature, a larger soil moisture at 700 than at 350 increases the relative rate of change in yield in elevated CO_2 . The increase in the elevated CO_2 effect at the summer cuts (Fig. 2) would thus result both from warmer air temperatures (Table 1) and (in 1993) from a larger soil moisture in elevated CO_2 during summer (Fig. 4).

An increased air temperature can favour the primary effect of CO_2 on photosynthesis, that is a reduction of photorespiration (reviewed by Long, 1991). The difference in CO_2 response between annual crops was attributed to differences in daily air temperature between growing seasons (Grashoff et al., 1995). Idso

Table 5. Relative rate of change in the dry-matter yield of the summer water limited (W-) swards in elevated CO₂ and at increased air temperature. The relative rates of change in yield (RY) were calculated as: $RY_{CO_2} = (Yield_{700} - Yield_{350})/Yield_{350}$ and $RY_{temp} = (Yield_{700+} - Yield_{700})/Yield_{700}$. The relative rates of change in yield were explained through a stepwise multiple regression. (T-T_c), is the difference between the mean daily air temperature (at 350 and 700 for the CO₂ and temperature effects, respectively) during regrowth (T, °C) and a constant T_c; (SM' - SM), is the difference in soil moisture between 700 and 350 and between 700+ and 700 respectively, for the CO₂ and temperature effects. The final equation is: $RY = a(T-T_c) + b(SM'-SM)$. The fitted values of a, b and Tc (± s.e.) are given

Relative rate of change in yield	Critical temperature (T _c , °C)	(T - T _c) coefficient a	(SM' - SM) coefficient b	
CO ₂	14.5±1	0.057±0.013	1.3±0.5	r ² = 0.68, p<0.00005
Temperature	18.5±1	-0.068±0.013	0.57±0.24	r ² =0.70, p<0.00005

et al. (1987) have shown that the stimulatory response of *D. carotta*, *Raphanus sativa* and *Gossypium hirsutum* was linearly related to air temperature. This interaction with temperature is consistent with information on individual plant species (Ackerly et al., 1992; Coleman and Bazzaz, 1992) and pasture communities (Newton et al., 1994). As both leaf emergence and leaf extension rates are strongly temperature dependent in perennial ryegrass, the potential for increased shoot growth in elevated CO₂ is presumably also temperature dependent, at least for sub-optimal air temperatures.

Above-ground dry-matter yield at increased air temperature in elevated CO₂

The mean (700 and 700+) air temperature during regrowth and the difference in soil moisture between 700+ and 700 accounted for 70% of the variability of the relative rate of change in yield at +3 °C in elevated CO₂ (Table 5). From this model, provided soil moistures at 700 and at 700+ are similar, the relative rate of change in yield at +3 °C in elevated CO₂ is positive for daily ambient air temperatures below 18.5 °C and negative otherwise. However, for a given air temperature, a lower soil moisture at 700+ than at 700 decreases the relative rate of change in yield at 700+, as compared to 700 (Table 5). This regression accounts for the positive effect of a +3 °C temperature increase on the DM yield under elevated CO₂ during early spring and at the last cut in autumn. It also accounts for the negative effect observed in summer (Fig. 2), due partly to a mean ambient air temperature above 18.5 °C and to the lower soil moisture at 700+, compared to 700 (Fig. 4).

The optimal air temperature for perennial ryegrass growth in ambient CO₂ is approximately of 20 °C (Ryle et al., 1992). Under our experimental conditions, this optimum does not seem to vary much in elevated CO₂, since the effect of a 3 °C increase in air temperature is positive below an ambient temperature of 18.5 °C and negative otherwise (Table 5). From these results, we might expect a somewhat complex interaction between elevated CO₂ and temperature increase effects on the productivity of cut perennial ryegrass swards: between 14.5 and 18.5 °C elevated CO₂ and temperature increase effects will act in synergy and above 18.5 °C the negative effect of temperature increase might offset the positive CO₂ effect.

Yet, such interactions with air temperature are likely to be affected by differences in soil moisture, as these can alter the sward growth in elevated CO₂ (Table 5), presumably through changes in the grass water potential. A larger soil moisture and, therefore, a higher water potential in elevated CO₂ tends to increase shoot growth, mainly by stimulating leaf extension and by increasing the specific leaf area (Van Loo, 1992). It is not clear, however, from the regression in Table 5, whether the differences in soil moisture content between the climatic treatments were of equal importance for low and for high soil moistures, or for low and for high evaporative demands.

Water use in elevated CO₂

In the present work, we have attempted to simulate the long-term impacts of climatic changes on the water use of perennial grass swards by supplying the same amount of water to all (W-) swards from the three climatic treatments. The control for irrigation was a

fully irrigated (W+ N+) sward at 350 and we had first assumed that the ET of the (N+) W- and W+ swards would be similar at the start of spring, that is when the soil moisture was close to the field capacity in both treatments. Nevertheless, in 1994, although the W+ swards were kept at the field capacity, their ET and their dry-matter yield were lower in spring and autumn than that of the N+W- swards (Fig. 7). This discrepancy indicates that the growth of the W+ swards was at some times restricted. At the field capacity, the oxygenation of the loamy soil used for this study might have been too low, resulting in the hypoxia of part of the root system and, hence (Jeffrey, 1988), in a restricted shoot growth. In a recent report, Adams and Akhtar (1994) have shown that the depletion of available nitrate through denitrification was the main reason for a decrease in growth of perennial ryegrass on waterlogged intact soil cores.

It is clear that the choice of the control for irrigation will affect water availability and may offset or enhance differences in soil moisture developing over the season. In the present study, the decline in soil moisture at W- occurred from May through September of both years, but was restricted at N- due to the lower ET of the N- swards (Table 3). With this seasonal pattern of soil water depletion, we observed during the growing season a small (-2%), but significant, decline in the average ET in elevated CO₂ (Table 3). A larger decline (-22%) in the daily ET of a C₄-dominated tallgrass prairie was recently reported by Ham et al. (1995). Nevertheless, as reviewed by Allen et al. (1990), most results with C₃ crops indicate a small, and sometimes non-significant (Kimball et al., 1994), elevated CO₂ effect on the ET per unit land area. A decline in the mean canopy evapotranspiration in elevated CO₂ results from a lower stomatal conductance (Morison, 1987; Nijs et al., 1989) and it would occur despite: i) the commonly observed increase in the foliage temperature (Idso et al., 1987; Morison and Gifford, 1984); ii) the increase of leaf area in high CO₂ during summer (Fig. 3), which was previously reported by Nijs et al. (1989).

We can expect that any reduction in ET in elevated CO₂ will translate into larger soil moisture content, or into increased drainage. Indeed, during the winter period, a significant increase in drainage (+9%, on average) was observed in elevated CO₂ (Table 3). Also, during the growing season in 1993, less soil water was used during spring in elevated than in ambient CO₂ (Table 3), which enhanced (Table 5) the positive effect of elevated CO₂ on DM yield during summer. Never-

theless, this seasonal pattern was not repeated in 1994, as, in this case, the decline in soil moisture at 700 (W-N+) closely paralleled that observed at 350. Under our experimental conditions, the soil water holding capacity was only a small fraction (approximately 7%) of the total water supplied by irrigation during one growing season. Therefore, the differences in soil moisture developing (or not) over the season were enhanced or offset by relatively small cumulative changes in the ET between CO₂ treatments.

The changes in WUE (+17, +30% at N- and +25, +26% at N+) under elevated CO₂ reported here are in good agreement with various studies in glasshouses and open topped chambers, which suggest seasonal WUE increases in elevated CO₂ from about +22 to +44% (Morison, 1993) and of +25% in the case of perennial ryegrass supplied with ample N fertilizer (Nijs et al., 1989). With wheat, in controlled environment experiments, the stimulation of WUE is no lower in water-stressed conditions than well-watered (André and Du Cloux, 1993; Gifford, 1979). Indeed, under our experimental conditions, the soil water deficit did not affect significantly the magnitude of the elevated CO₂ effect on the water use efficiency of the ryegrass swards (Fig. 7C).

Water use at increased air temperature in elevated CO₂

The increase in VPD at 700+ compared to 700 (Table 1), by increasing the evaporative demand, obviously contributed to the observed (Table 3) increase in ET in the combined elevated CO₂ and temperature treatment. Nevertheless, as the average ratio between ET and VPD was significantly lower at 700+ than at 700 (-19%, data not shown), the canopy conductance for water vapour was apparently lowered at +3 °C in elevated CO₂.

With field-grown perennial ryegrass, the threshold for stomatal response to leaf water potential is approximately at -1.0 MPa (Jones et al., 1980). From Figure 5, it can be seen that, on average, when the soil moisture was below 15% of the field capacity, the pre-dawn leaf water potential became lower than -1 MPa. Therefore, at N+W-, the lower soil moisture content (Fig. 4) at 700+ presumably led to some degree of stomatal closure, thereby restricting the ET. Interestingly, although the average annual WUE was not modified by the 3 °C temperature increase (Table 4), the product between WUE and VPD (see Jones, 1992, p. 280) was larger at 700+ than at 700, which indicates that water was

used more efficiently at 700+ than would be expected, given the increase in VPD.

Both during the winter period and during the growing season drainage was significantly reduced (by -29 to -34%, Table 3) by a supplemental 3 °C in elevated CO₂. In case of a 3 °C global warming, this reduction in drainage (by 22 to 30% at 700+ compared to 350) would clearly affect the water fluxes at the field, catchment and ecosystem scale.

Taken together, these results show that, in field-like conditions, changes in the soil water status will interact little with the direct effects of elevated CO₂ on grass above-ground productivity if the air temperature is not modified. However, in case of a combined increase in CO₂ concentration and in air temperature, the faster depletion of soil water during spring might lead to an earlier onset of the soil water deficit in summer. Interestingly, the potential for a large positive effect of elevated CO₂ on DM yield at higher air temperatures during summer is not likely to be reached in case of a combined +3 °C temperature increase, due to the negative effects of low soil moistures and above-optimal temperatures on the productivity of temperate grasses. On the other hand, the extension of the growth period in early spring and late autumn at + 3 °C could well compensate for a depression in DM yield during summer. Yet, in early spring and late autumn the effects of elevated CO₂ on the above-ground productivity are likely to be minimized, due to the low air temperatures.

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