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Seasonal changes in the response of winter wheat to elevated atmospheric CO_2 concentration grown in Open-Top Chambers and field tracking enclosures

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Abstract

Winter wheat was grown at ambient and elevated (ambient plus 350 $\mu L\,L^{-1}$) CO_2 concentrations in open top chambers and in field-tracking sun-lit climatized enclosures (elevated is 718 $\mu L\,L^{-1}$). There was no significant effect of CO_2 concentration on sheath, leaf and root biomass and leaf area in the early spring (January to April). 24-h canopy CO_2 exchange rate (CCER) was not significantly affected either. However, elevated CO_2 concentration increased CCER at midday, decreased evapotranspiration rate and increased instantaneous water-use-efficiency during early spring. Leaf, sheath and root nitrogen concentration per unit dry weight decreased and nonstructural carbohydrate concentration increased under elevated CO_2, and N-uptake per unit ground area decreased significantly (–22%) towards the end of this period.

These results contrast with results from the final harvest, when grain yield and biomass were increased by 19% under elevated CO_2. N concentration per dry weight was reduced by 5%, but N-uptake per unit ground area was significantly higher (+11%) for the elevated CO_2 treatment. 24-h and midday-CCER increased significantly more in late spring (period of 21 April to 30 May) (respectively by +40% and 53%) than in the early spring (respectively 5% and 19%) in response to elevated CO_2. Midday evapotranspiration rate was reduced less by elevated CO_2 in the late spring (–13%) than in early spring (–21%). The CO_2 response of midday and 24-h CCER decreased again (+27% and +23% resp.) towards the end of the growing season.

We conclude that the low response to CO_2 concentration during the early spring was associated with a growth-restriction, caused by low temperature and irradiance levels. The reduction of nitrogen concentration, the increase of nonstructural carbohydrate, and the lower evapotranspiration indicated that CO_2 did have an effect towards the end of early spring, but not on biomass accumulation. Regression analysis showed that both irradiance and temperature affected the response to CO_2.

Keywords: CO_2–light interaction, CO_2–temperature interaction, early spring growth, Open-Top Chambers, seasonal influences, *Triticum aestivum*, wheat, yield

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Introduction

During the last decade, a steady flow of information on the responses of plants to increased atmospheric CO_2 concentration has become available. Studies have shifted from experiments in controlled-environment chambers with artificial lightning, to conditions more resembling field conditions (Open-Top Chambers – OTCs, field-tracking sun-lit enclosures and free air CO_2 enrichment experiments; Allen *et al.* 1992). Recent studies have emphasized the interaction between CO_2 and the phenology of the crop (Newton *et al.* 1994; Dijkstra *et al.* 1996; Grashoff *et al.* 1996). The latter modelling study showed that a later sowing date resulted in a higher relative response to CO_2 concentration due to higher growth temperatures. A study by Butterfield & Morison (1992), using a historical weather data set, did not show a

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consistent effect of average temperature on the developmental rate of winter wheat in the UK. They concluded that more detailed studies on the effects of the temperature during the growing season were needed.

The mechanism for CO_2–temperature interaction on photosynthesis is well understood: at the site of the Rubisco enzyme, the ratio of carboxylation to oxygenation is dependent on the ratio of the concentrations of CO_2 and O_2. Increased CO_2 concentration results in higher photosynthesis through suppression of photorespiration and increased CO_2 availability. Low temperature increases the CO_2/O_2 specificity factor of Rubisco, and the solubility ratio of CO_2 to O_2 (Jordan & Ogren 1984). Therefore, higher CO_2 concentration increases photosynthesis less at low than at high temperatures (Bowes 1991).

Independent of the effects on photosynthesis, temperature has a strong effect on growth. Growth is more sensitive to temperature than photosynthetic rate is. Temperature determines leaf area expansion early in the season for winter wheat (Spitters *et al.* 1989) and *Lolium perenne* (Davies *et al.* 1989; Nijs & Impens 1996), irrespective of light intensity. A higher leaf photosynthetic rate will therefore not automatically lead to a greater biomass accumulation at low temperatures. Consequently the CO_2 response for productivity is limited within the narrow boundaries of temperature-determined leaf area development.

The present study deals with changes in the response to elevated CO_2 concentration during the course of a growing season for a winter wheat crop under semifield conditions. Data are presented from repeated harvests of plants grown in OTCs and gas exchange of plants grown in field-tracking sun-lit crop enclosures.

Materials and methods

Open-Top Chambers

OTCs were constructed as equilateral hexagons with sides of 0.87 m, height of 1.90 m, and a volume of 3.7 m^3. Ground area inside the OTC was 1.95 m^2. OTCs were made of 3 mm thick polycarbonate (Lexan). A frustum of 25 cm at an angle of 45° was part of the OTC. Air was blown into the OTC by means of a blower (for more information see Dijkstra *et al.* 1995). The number and positions of the air-inlets ensured a horizontally uniform microclimate, while an air-inlet above the canopy further homogenized the microclimate through strong turbulence. Air replacement rate was 3.6 chamber volumes per minute. Pure CO_2 was added to the ambient air just before the blower inlet and thoroughly mixed. CO_2 concentration was manually controlled and adjusted when needed. Temperature (measured with shielded thermocouples) and CO_2 concentration were recorded automatically (HP-7500 data-acquisition device) at 30 cm above the soil surface.

The OTC experiment consisted of 18 plots, six ambient, six elevated (plus 350 $\mu L\,L^{-1}$) OTCs and six nonchambered control plots (for agronomic comparison only). Nine plots (3 ambient, 3 elevated and 3 control), located on one side of the field were used for a destructive growth analysis. This experiment was done in 1992/1993 and repeated in 1993/1994. These experiments ended on 23 March 1993 and 21 April 1994, respectively. The plots on the other half of the field were left undisturbed until final harvest. All plots were otherwise similarly treated.

Before the 1992/1993 experiment, the top 30 cm of the soil was replaced with a light clay ('Polder' vague soil; de Bakker & Schelling 1966), silty clay (45% smaller than 2 μm), containing 3% organic matter and about 10% $CaCO_3$, pH 7.5–8.0. Border rows around the OTC, or around a similar area in the open plots, were at least 30 cm wide. Plots were supplied with a drip-irrigation system to ensure optimal water supply.

Averaged CO_2 concentration in 1993/1994 was 374 $\mu L\,L^{-1}$ for the ambient treatment and 706 $\mu L\,L^{-1}$ for the elevated treatment. During the winter period the difference was 401 $\mu L\,L^{-1}$ for ambient OTCs, vs. 718 $\mu L\,L^{-1}$ for elevated CO_2. Irradiance (Q, 400–700 nm) reduction by the chamber walls was on average 25% over the light period as compared to the outside. Daily mean temperature inside the OTCs was on average 2.9 °C higher than outside with a daily maximum difference of 4.7 °C. Although temperatures were higher in the OTC as compared with the outside environment, frost did occur inside the OTCs. More information on the OTC facility is available from Dijkstra *et al.* (1995). The experiment was analysed as a completely randomized design ($n = 3$) using ANOVA. Weight and areas were analysed after LN transformation. Statistical evaluation was done at $P < 0.05$.

Field-tracking sun-lit climatized enclosures

In addition to the OTC experiment, winter wheat was grown in four individually climatized crop enclosures, surrounded by border plants in 1992/1993. The enclosures were part of the Wageningen Rhizolab Facility, a joint research facility of the AB-DLO Institute and the Wageningen Agricultural University (Van de Geijn *et al.* 1993,1994).

The crop enclosures (1.25×1.25 m^2 by 1.15 m high) were made of 5 mm thick polycarbonate. The temperature in the enclosures followed the outside temperatures, as registered by a weather station associated with the Rhizolab, by computer controlled air-conditioning. The enclosures were semiclosed circulation systems with a recirculation rate of 800 $m^3\,h^{-1}$ and a fresh ambient air

injection of about $50\,m^3\,h^{-1}$. CO_2 concentration and dewpoint of the replacement air and chamber air, and air replacement flow rates were monitored every 10 and 20 min, respectively. In addition, the condensate formed within the air conditioner was measured using a tipping-bucket rain gauge, as part of the total water-balance. Part of the air was forced into the soil by a slight over-pressure in the chamber and recovered in an air-drain, placed in the soil at 15 cm depth. This prevented air within the soil compartment from entering the canopy compartment. The soil compartment was filled with the same soil as in the OTC experiment, to a depth of 1.80 m.

Nutrients and water were given in optimal amounts according to agricultural guidelines. Nitrogen in the soil solution was measured regularly to ensure enough N was available for growth, soil water content was measured with time frequency reflectometry (Van de Geijn *et al.* 1994). Average CO_2 concentration was 360 and $718\,\mu L\,L^{-1,}$ respectively, in the ambient and elevated treatment (2 enclosures each). Root density was observed with mini-rhizotrons at a depth of 5, 10, 15, 20, 30, 45, 60, 80 and 100 cm, but only the data of the root density at 5 cm depth are presented. Video images were digitized for root counts.

Experimental procedures

Experimental procedures for the Rhizolab experiment and the OTC for both 1992/1993 and 1993/1994 were comparable. In the following section, the procedures for the 1993/1994 OTC experiment are described. Winter wheat (*Triticum aestivum* cv. Ritmo) was sown by hand on 25 and 26 October 1993. Row spacing was 12.5 cm and interplant spacing was 2.2 cm (360 plants m^{-2}). The OTCs were placed the following day. At emergence (2/11/93), CO_2 addition started. Diseases and pests were controlled when required. When plants reached the second node stage (decimal code 32), CCC (Terpal) was applied to the plants in the OTCs, but not to the plants in the Rhizolab.

CO_2 addition was stopped on 26 July after ripening of the seed and complete senescence of all plant parts in the chambers. Final harvest took place on 8 August 1994 for the chambered plots, and on 22 August for the open field plots. Harvest date for the climatized enclosures was 9 August 1993.

In both years several harvests were taken in early spring (January until April). The experiment in 1992/1993 lasted until 23 March, while that in 1993/1994 was extended until 21 April . At each harvest, four 25-cm row lengths were taken from each plot and the plants were separated in leaf blades, sheaths and roots. At final harvest, four representative samples (four 25 cm row lengths) per OTC (1992/1993) or the whole chamber was analysed (1993/1994 and Rhizolab). These plants were separated in leaf blades, stem (plus leaf sheaths), chaff and grain and stubble (part of the stems that remained after the plants were cut off at ground level, and excluding any roots). Number of tillers was counted and thousand grain weight determined. Dry weight was obtained after drying for at least 24 h at 70 °C. The samples were later used to analyse soluble sugar, starch, C and N-content.

Chemical composition

Total soluble carbohydrates were extracted by boiling for 10 min in demineralized water. Reducing monosaccharides were determined in the extract by titration with sodium thiosulphate (Williams 1984). Starch was enzymatically hydrolysed for 1 h at 60 °C by amyloglucosidase (EC 3.2.1.3; Merck, Darmstadt BRD). Subsequently, the glucose formed was determined by titration (Williams 1984). Starch concentration was expressed as mg CH_2O g^{-1} dry weight. Total nitrogen was determined by CHN analysis on a Heraeus CHN-rapid (Hanau-Germany).

Analysis of Rhizolab data

Canopy CO_2 exchange (CCER) and evapotranspiration rate were calculated from the difference between CO_2 and H_2O vapour concentrations of the replacement air and the chamber air, multiplied by the flow rate. Water vapour was measured with dew-point hygrometers (series 3000, Michel Instruments Ltd, Cambridge, England), and CO_2 with infra-red gas analysers (Uras 10E, Hartman and Braun, Frankfurt, Germany). CO_2 was corrected for dilution by water vapour, and evapotranspiration included the condensate from the air conditioner. Gas exchange measurements, weather data and all other variables were converted into hourly means. On some days, the measurement of gas exchange was disturbed by opening the enclosures for a short time for nondestructive observations, weeding and spraying against diseases and pests. These days were excluded from the analysis.

The CO_2 response ratio (R, ratio of elevated over ambient values of a certain parameter) was calculated for the 24-h and midday CCER, and for the midday evapotranspiration and instantaneous water use efficiency (WUE) for three periods during the growth season. These periods were days 50 (19 February) to 110 (22 April), called 'early spring'; days 111 (23 April) to 150 (30 May), called 'late spring'; and days 151 (31 May) to 221 (9 August, harvest), called 'summer'.

The additive and multiplicative effects of irradiance and temperature on R, the CO_2 response ratio, for different parameters was estimated with multiple regres-

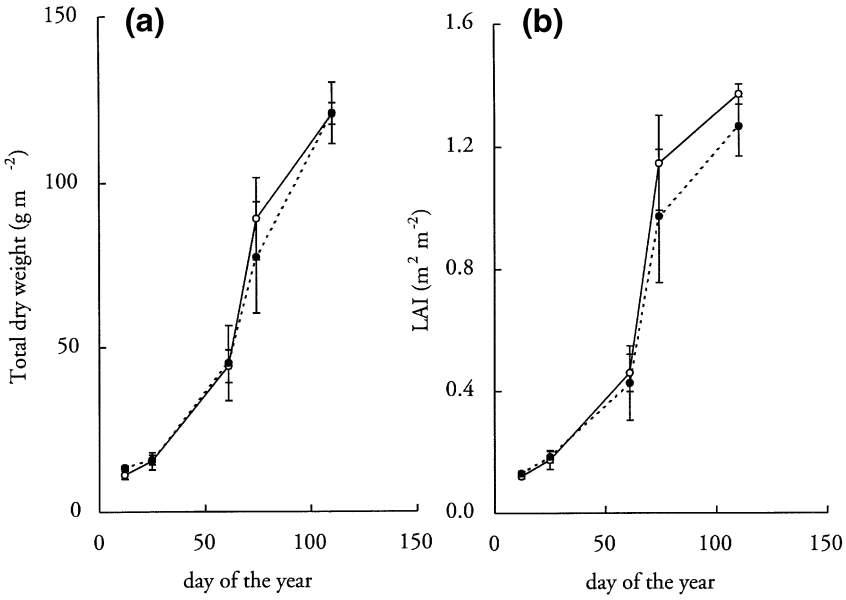

Fig. 1 Total plant dry weight (a, g m^{-2}) and leaf area index (b, m^2 m^{-2}) at ambient (open squares) and elevated CO_2 concentration (closed squares) against DOY (day of year). Values ± 1 SE ($n = 3$).

sion for the entire spring (days 50–150). Daily averaged values for the CO_2 response ratio were related to daily averaged values of temperature and irradiance level. Likewise, midday R was related to midday temperature and irradiance. The regression equation was, $R = b_1 + b_2 * T + b_3 * Q + b_4 * T * Q$, where T is the temperature (either midday or daily average in°C) and Q is the short-wave radiation (Q at midday in MJ m^{-2} h^{-1} or as daily sum in MJ m^{-2} d^{-1}), and b_1, b_2, b_3 and b_4 are regression coefficients. The summer period was excluded because it deviated from relationships derived for the other periods, due to values during the last weeks of the summer. This was probably caused by a direct effect of CO_2 concentration on leaf senescence. Stepwise multiple regression indicated that the first significant factor in the equation was the interaction between Q and T (data not shown).

Results

Early spring growth

CO_2 increase had no effect on total plant biomass per unit ground area during early spring (January until April) in the 1992/1993 (results not shown) nor in the 1993/1994 experiment (Fig. 1a). Leaf area index (LAI) of the elevated treatment decreased by 8% (NS) at the last two harvests as compared to the ambient treatment (Fig. 1a). A nonsignificant decrease in LAI was also observed on 23 March for the 1992/1993 experiment. CO_2 did not significantly alter leaf or sheath dry weight or leaf area per plant in 1993/1994 (Table 1). Root dry weight showed significant increases on 26 January and 21 April (Table 1). Root density at 5 cm depth was significantly higher after 18 March (Fig. 2) under elevated CO_2, corresponding to significant root weight increases from 16 March onwards (in, respectively, the Rhizolab and the OTC experiment in 1992/1993, data not shown). Leaf weight ratio (leaf dry weight divided by total plant dry weight) and specific leaf area (ratio of leaf area and leaf dry weight) were not affected by elevated CO_2 (Table 1). Rate of development was not affected either (results not shown).

The C-content of the plant material was not altered, but a significant CO_2–time interaction was found for the N-content and soluble sugar content of leaves and sheath. Towards the end of early spring, leaf and sheath N-content were decreased while soluble sugar content increased under elevated CO_2 (Table 2).

N-uptake per unit ground area for the crop grown under elevated CO_2 was 22% lower than for the ambient treatment on 21 April (DOY 110, Fig. 3). A similar reduction (18%) was found in the 1992/1993 season (1.56 and 1.28 g N m^{-2} ground area on March 23 (DOY 82) for ambient and elevated CO_2, respectively). The N content per unit leaf area was also reduced by elevated CO_2 (12%, based on Tables 1 and 2).

Final harvest

In contrast to growth in early spring, CO_2 concentration significantly stimulated grain yield and total above-ground biomass by 19% (Table 3). This was in line with that found for 1992/1993 in the OTCs (14%), and measured in the Rhizolab experiment (24%). Stem, leaf, stubble and chaff dry weights were also increased. The increased grain yield was mainly associated with a

Table 1 Dry weight (mg plant^{-1}) of leaf blade, sheath and root, leaf blade area (cm^2 plant^{-1}), SLA (m^2 leaf area kg^{-1} leaf dry weight) and LWR (g leaf dry weight g^{-1} plant dry weight) as affected by CO_2 concentration. SE is standard error ($n = 3$)

	DOY	13-Jan-94 13		26-Jan-94 26		3-Mar-94 62		16-Mar-94 75		21-Apr-94 110	
CO_2 concentration		mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
Ambient	leaf	19.79	(1.16)	30.21	(1.42)	82.65	(7.96)	197.3	(24.1)	214.0	(23.6)
	sheath	10.96	(0.72)	16.70	(1.80)	52.13	(3.93)	88.30	(5.93)	123.3	(8.9)
	root	6.00	(0.05)	6.83	(0.53)	16.71	(1.15)	32.62	(4.80)	45.75	(3.96)
	leaf area	3.93	(0.16)	6.03	(0.38)	15.60	(1.59)	40.79	(1.78)	43.68	(5.05)
	SLA	19.60	(1.52)	20.00	(0.31)	18.72	(0.33)	21.16	(1.48)	20.35	(0.20)
	LWR	0.534	(0.005)	0.562	(0.004)	0.546	(0.007)	0.617	(0.017)	0.557	(0.016)
Elevated	leaf	21.02	(0.97)	31.16	(1.48)	84.05	(11.4)	151.6	(23.0)	231.7	(15.7)
	sheath	13.06	(0.63)	15.34	(0.34)	52.78	(6.60)	92.51	(7.89)	128.7	(11.3)
	root	6.37	(0.38)	9.06	(0.56)	17.51	(1.05)	33.53	(6.38)	64.30	(5.50)
	leaf area	3.92	(0.24)	6.42	(0.08)	14.52	(2.24)	34.72	(4.77)	44.47	(3.11)
	SLA	18.78	(1.09)	20.76	(0.97)	17.09	(0.40)	22.99	(0.45)	19.19	(0.45)
	LWR	0.517	(0.010)	0.559	(0.010)	0.542	(0.011)	0.543	(0.012)	0.546	(0.009)

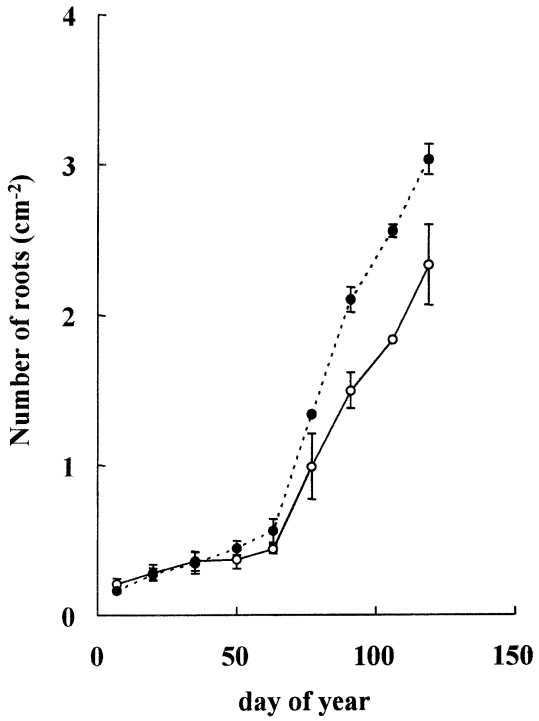

Fig. 2 Number of roots per cm^{-2} as observed with mini-rhizotrons at a depth of 5 cm in the Rhizolab experiment at ambient (open squares) and elevated (closed squares) CO_2 concentration against day of year. Values ± 1 SE ($n = 3$).

higher number of ear-bearing tillers and only slightly by other factors such as an increased number of seeds per ear and seed weight. Stem weight per tiller was also increased. Plant development was not affected.

The C- and N-content were affected by elevated CO_2 concentration, but not consistently for all plant parts (Table 4). Especially noteworthy was the absence of a decreased grain N-content. Soluble sugar and starch concentrations were not significantly affected by CO_2 concentration.

N concentration per total plant dry weight was reduced by 5% (14.4 vs. 13.7 mg g^{-1}). However, in contrast to the spring, the N-uptake per unit ground area at final harvest was actually increased significantly by 11% (32.2 and 35.6 g m^{-2} for ambient and elevated, respectively).

Gas exchange characteristics

During the early spring, plant dry weight and leaf area (Fig. 1, Table 1), 24-h and midday CCER (per unit ground area) (Fig. 4a,b) and evapotranspiration rates (Fig. 5a) increased. However, the effect of CO_2 on the 24-h CCER during this period was small, as evidenced from the CO_2 response ratio (average ratio of elevated over ambient treatment, Fig. 4c). This CO_2 response ratio for 24-h CCER was 1.05 (± 0.03) during early spring (not significantly different from one) and significantly increased to 1.40 (± 0.03) in the late spring, and then decreased again to 1.23 (± 0.03) during the summer.

The CO_2 response ratio for midday CCER was 1.19 ± 0.03 (Fig. 4d) for early spring, significantly different from one. The relative response then increased significantly during late spring to 1.53 (± 0.04) and decreased again (1.27 ± 0.03) during the summer.

Table 2 C-content, N-content and soluble sugar content (mg g^{-1} dry weight) of leaf blade, sheath and root as affected by CO_2 concentration. SE is standard error ($n = 3$)

	DOY		13-Jan-94 13		26-Jan-94 26		3-Mar-94 62		16-Mar-94 75		21-Apr-94 110	
	CO_2 concentration		mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
C-content	ambient	leaf	440	(1)	440	(1)	445	(1)	432	(5)	430	(1)
		sheath	406	(1)	401	(1)	406	(3)	397	(6)	407	(3)
		root	393	(14)	380	(16)	403	(6)	376	(9)	398	(11)
	elevated	leaf	443	(1)	438	(2)	442	(2)	433	(0)	426	(3)
		sheath	405	(4)	404	(2)	405	(7)	403	(3)	414	(2)
		root	379	(16)	379	(18)	401	(5)	381	(5)	404	(8)
N-content	ambient	leaf	55.2	(0.3)	55.2	(0.8)	51.5	(1.0)	48.7	(1.4)	41.3	(0.4)
		sheath	43.5	(0.6)	44.5	(0.6)	41.1	(2.0)	40.2	(3.1)	32.0	(1.7)
		root	23.1	(0.1)	22.7	(0.6)	25.5	(0.4)	17.6	(1.0)	17.2	(0.8)
	elevated	leaf	54.7	(0.4)	54.6	(0.4)	44.8	(1.7)	43.4	(2.4)	34.4	(1.2)
		sheath	41.9	(0.7)	46.9	(0.9)	35.0	(2.0)	33.4	(2.3)	23.5	(1.6)
		root	21.0	(0.9)	21.0	(1.7)	22.3	(1.5)	16.0	(1.1)	14.0	(1.2)
Sol. sugars	ambient	leaf	37.1	(1.4)	39.7	(2.4)	105.7	(4.3)	52.0	(5.2)	124.0	(7.5)
		sheath	68.6	(1.6)	71.8	(0.2)	169.7	(9.1)	84.7	(16.3)	209.0	(7.8)
		root	55.8	(4.8)	44.7	(3.2)	80.4	(4.4)	20.2	(8.3)	66.3	(5.3)
	elevated	leaf	44.0	(1.0)	42.6	(2.5)	170.0	(18.0)	89.0	(5.4)	179.0	(18.8)
		sheath	76.1	(5.7)	71.1	(3.6)	224.7	(34.9)	137.7	(12.0)	304.3	(22.4)
		root	54.9	(1.0)	44.0	(4.2)	85.8	(4.5)	77.5	(38.8)	88.4	(5.3)

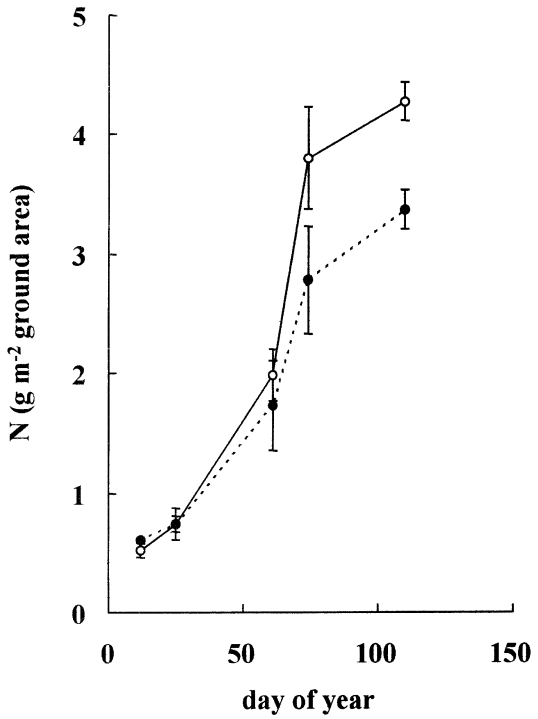

Fig. 3 N-content (g m^{-2} ground surface) at ambient (open squares) and elevated (closed squares) CO_2 concentration against day of year. Values ± 1 SE ($n = 3$).

Evapotranspiration rate was reduced by elevated CO_2 (Fig. 5). The average response ratio for the evapotranspiration during early spring was 0.79 (± 0.03), significantly lower than during late spring (0.87 ± 0.01) and the summer (0.90 ± 0.02). The difference between late spring and summer was not significant. Apparently, in the later stages of growth, the effect of elevated CO_2 on canopy C-fixation rate per unit ground area was declining, while that on evapotranspiration persisted (Figs 4d and 5b).

As a consequence, water use efficiency (WUE) was higher for the elevated CO_2 treatment (Fig. 5c). The response ratio of WUE increased significantly from early spring (1.56 ± 0.05) to late spring (1.77 ± 0.04), but significantly declined again later in the season (1.47 ± 0.07).

The increase of CCER to CO_2 concentration, and the reduction of evapotranspiration from spring to summer coincided with increases in irradiance levels and temperatures. By means of multiple regression, the effects of irradiance and temperature on the relative response ratio can be separated and quantified (Table 5, Fig. 6).

There was a significant interaction between irradiance level and temperature: the higher the irradiance and temperature level, the greater the response of the 24-h

CCER to CO_2 concentration (Fig. 7a). The response of midday CCER to irradiance and temperature showed a similar interaction, except that for temperatures below 6 °C the response to CO_2 concentration seemed to decrease when irradiance level increased (Fig. 7b).

Discussion

Grain yield and biomass responses to CO_2 concentration

The responses of grain yield and biomass to CO_2 concentration in this study (14–24%) were comparable to results from other experiments with winter wheat (Havelka *et al.* 1984; Mitchell *et al.* 1993; Chaudhuri *et al.* 1990, Wheeler *et al.* 1996a) and simulation studies (Goudriaan & Unsworth 1990). Higher numbers of ears and seeds per ear contributed to the increased yield (Wheeler *et al.* 1996a and this study for winter wheat; Dijkstra *et al.* 1996 and Mulholland *et al.* 1997 for spring wheat), although effects of CO_2 on seed weight were sometimes found (Wheeler *et al.* 1996a). Wheeler *et al.* (1996b) showed that CO_2 concentration did not affect the seed-filling rate per seed for winter wheat.

The reduction of the N-concentration per unit dry weight under elevated CO_2 was 5% at final harvest (Table 4). However, total N-uptake per unit ground area was increased, as found for potato (7%) and faba bean (11%, Dijkstra *et al.* 1995). A reduction of the N-concentration per unit dry weight as a result of CO_2 elevation is often found in plants (Kuehny *et al.* 1991; Poorter *et al.* 1997). In this experiment it was accompanied by a rise in the sugar concentration (Table 2), but not fully explained by it. A higher concentration of soluble sugar and/or starch is also a general finding under elevated CO_2 concentration (Kuehny *et al.* 1991; Poorter *et al.* 1997), and may (Acock *et al.* 1990), or may not (Yelle *et al.* 1989a,b; Baxter *et al.* 1995) result in end-product inhibition. High concentration of soluble sugars can also reduce gene-expression for a number of proteins involved in photosynthesis (Nie *et al.* 1995). Reductions in Rubisco and associated proteins were related to a reduction in N-content and photosynthesis (Van Oosten *et al.* 1992; Jacob *et al.* 1995; Li *et al.* 1998).

Table 3 Total biomass, grain yield, harvest index, stem (plus sheath), leaf blade, stubble and chaff dry, number of seed-bearing ears, and number of seeds per ear and thousand grain weight as affected by CO_2 concentration in the 1993/1994 OTC experiment. Weights are in g m^{-2}, harvest index as a ratio of grain yield to total above ground biomass, number of ears are per m^2, thousand grain weight as g 1000 $seeds^{-1}$. SE is standard error ($n = 3$)

	Ambient	SE	Elevated	SE
Total biomass	2302	(11)	2709	(41)
Grain yield	1009	(7)	1206	(11)
Harvest index	0.437	(0.003)	0.443	(0.003)
Stem dry weight	579	(5)	678	(7)
Leaf dry weight	362	(13)	409	(13)
Stubble dry weight	94.4	(1.1)	109	(4.5)
Chaff	200	(1.4)	235	(3.1)
n of ears	669	(6)	756	(6)
n seed ear^{-1}	34.3	(0.4)	35.5	(0.8)
Thousand grain weight	40.8	(0.4)	42	(0.8)

Early season biomass response to CO_2 concentration

CO_2 did not stimulate biomass or leaf area during early spring (January until end of April), even though at the

Table 4 C, N, soluble sugar and starch content (mg g^{-1} dry weight) of stubble, stem (plus sheath), leaf blade, chaff and grain, at final harvest in OTCs as affected by CO_2 concentration. n.d. not determined, SE is standard error ($n = 3$)

CO_2 concentration		Stubble	SE	Stem	SE	Leaf	SE	Chaff	SE	Grain	SE
C-content	ambient	412	(4)	428	(1)	379	(2)	401	(1)	451	(6)
	elevated	418	(1)	429	(0)	389	(4)	410	(0)	457	(0)
N-content	ambient	10.5	(0.3)	6.17	(0.34)	9.43	(0.44)	6.10	(0.09)	22.9	(0.2)
	elevated	8.96	(0.25)	5.28	(0.12)	7.88	(0.48)	5.55	(0.09)	22.4	(0.3)
Sol. sugars	ambient	9.35	(1.46)	12.2	(0.5)	22.0	(0.6)	20.6	(0.3)	37.9	(1.1)
	elevated	7.72	(0.54)	10.9	(0.6)	22.8	(0.7)	19.4	(0.8)	38.0	(0.9)
Starch	ambient	3.90	(0.44)	n.d.		2.78	(0.18)	14.8	(0.4)	704	(10)
	elevated	4.22	(0.18)	n.d.		4.45	(1.28)	16.4	(1.3)	716	(11)
C/N ratio	ambient	40.2	(0.7)	71.5	(3.4)	41.5	(1.7)	67.1	(0.8)	19.7	(0.2)
	elevated	48.6	(1.6)	84.5	(2.3)	49.9	(3.3)	75.2	(1.1)	20.5	(0.3)

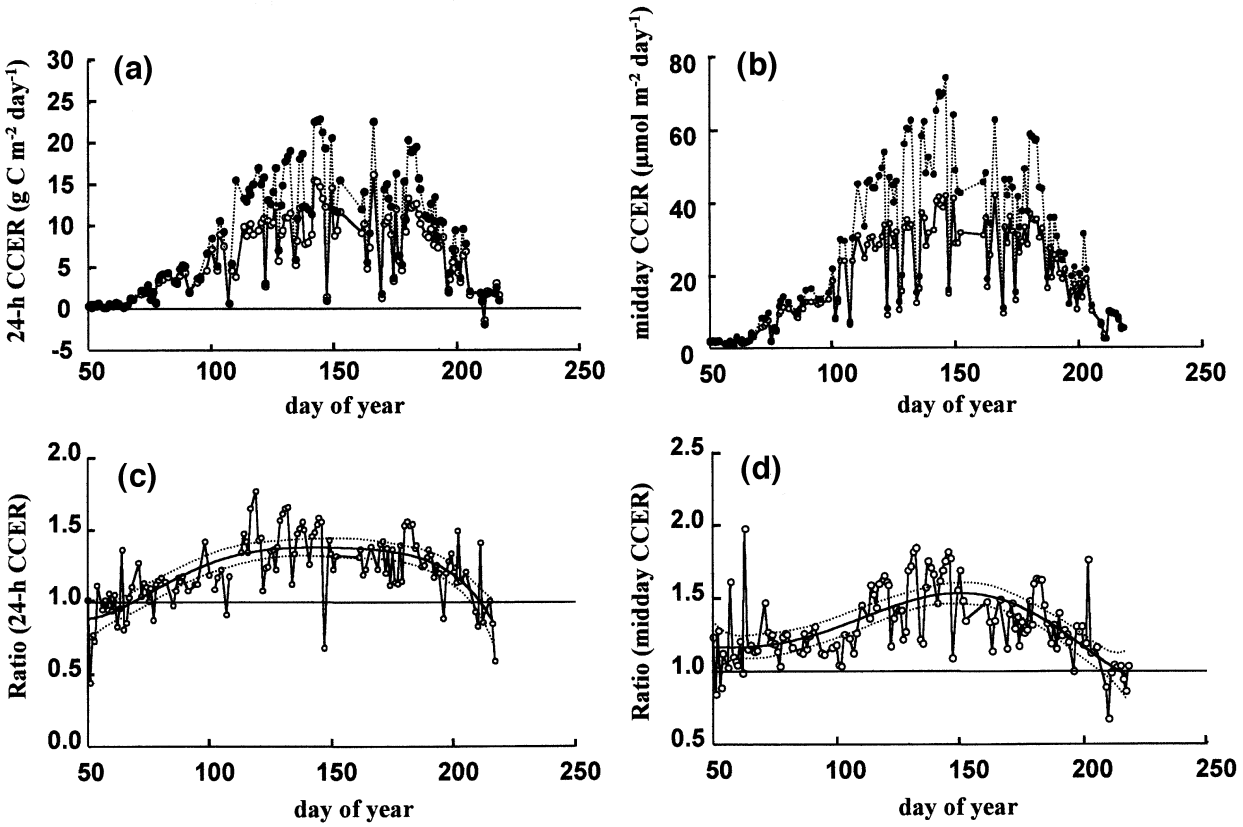

Fig. 4 24-h averaged (a, g C m^{-2} d^{-1}) and midday (b, µmol $m^{-2}s^{-1}$) canopy CO_2 exchange rate (CCER) of winter wheat at ambient (open squares) and elevated (closed squares) CO_2 concentration and the ratio of elevated CO_2 over ambient CO_2 concentration treatment for the 24-h CCER (c) and midday CCER values (d) against day of year. Regressions lines with 95% confidence interval (panels c and d) are based on 5th-order polynomial.

end of the growing season a substantial and significant increase in biomass was found (Fig. 1, Table 3, References cited above). Similarly *Lolium perenne* did not show a shoot growth response to CO_2 concentration under winter conditions (Newton *et al.* 1994; Schapendonk *et al.* 1997), or even a reduction under elevated CO_2 by 28% (Nijs & Impens 1996). Increasing the winter temperature by 4 °C increased the response of the harvestable biomass to elevated CO_2 from −28% to +14% (Nijs & Impens 1996).

A low growth response to elevated CO_2 concentration under low temperatures is predicted by crop growth models (Schapendonk & Brouwer 1985; Grashoff *et al.* 1996). Davies *et al.* (1989) and Spitters *et al.* (1989) showed that growth during these periods is sink-limited, and will only increase when temperatures rise and stimulate sink activity. CO_2 concentration apparently was not able to break this sink-limitation (Nijs & Impens 1996 for *Lolium perenne* and this study for winter wheat).

At the beginning of early spring, CO_2 concentration did not have an effect on either the biomass or leaf area or on N and sugar content. However, towards the end of early spring, CO_2 concentration did reduce the nitrogen concentration and increased the concentration of soluble sugars (Table 2, Fig. 3), even though growth still did not change under influence of the elevated CO_2. The combination of a lower N-concentration and unchanged biomass resulted in a reduction of the N-uptake per unit ground area of 22%. *Lolium perenne* showed similar reduction of N per unit ground area in response to CO_2 and winter conditions, up to 69% (estimated from Nijs & Impens 1996). Likewise, a CO_2–temperature interaction (20–35 °C) effect on N-content of *Pascopyrum smithii* (Read & Morgan 1996) and soybean (Bunce & Ziska 1996) showed that the lower the temperature the greater the reduction in nitrogen content by CO_2 concentration.

This temperature–CO_2 interaction on N-content is contrary to what is expected for Rubisco content: the lower the temperature, the lower the effect of CO_2 on photosynthesis, the smaller the reduction in Rubisco content is expected to be (Woodrow 1994). A tempera-

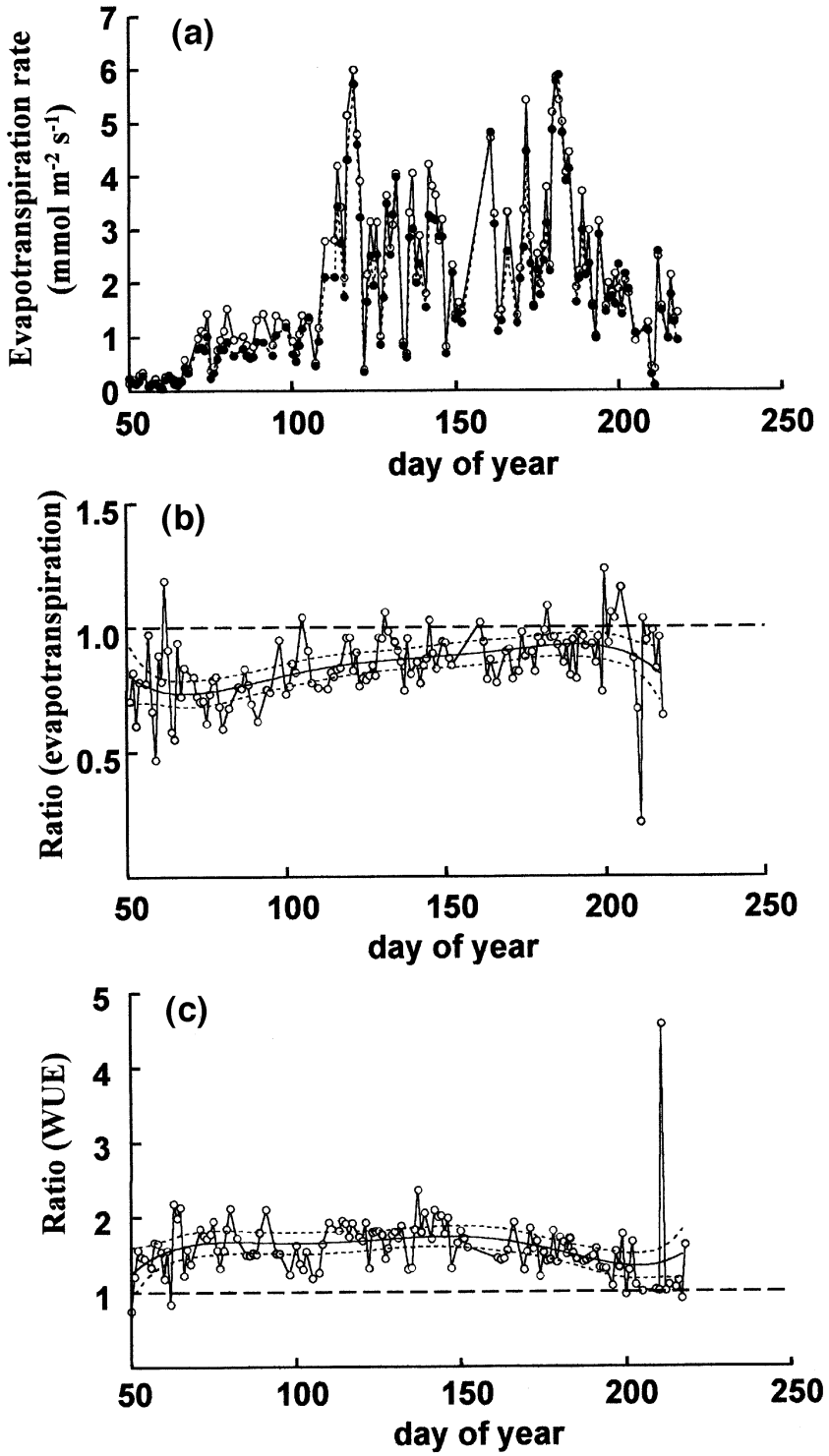

Fig. 5 Midday canopy evapotranspiration rate (mmol $m^{-2}s^{-1}$) of winter wheat at ambient (open squares) and elevated (closed squares) CO_2 concentration (a) and the ratio of elevated CO_2 over ambient CO_2 concentration treatment for evapotranspiration rate (b) and the instantaneous water use efficiency (c). Regressions lines with 95% confidence interval (b,c) are based on 5th-order polynomial.

ture-limited sink capacity might be involved, either directly on the nitrogen uptake or indirectly on the nitrogen use.

The first indication of a growth response to CO_2 elevation was found as an increase in root density (Fig. 2) and root weight (see text).

Ecosystem gas exchange

Early spring conditions in the Netherlands are characterized by low temperatures and low light intensities. A low response to CO_2 concentration at low temperatures is predicted from knowledge of the basic processes of

Table 5 Regression coefficients b_1 to b_4 of the regression equation relating the CO_2 response ratio of the 24-h CCER to 24-h temperature and irradiance (MJ m^{-2} d^{-1}) (A) and the response ratio of the midday CCER to midday T and irradiance levels (MJ m^{-2} h^{-1}). Regression equation is $R = b_1 + b_2 * T + b_3 * Q + b_4 * T * Q$

	24-h	midday
b_1	0.8929	1.3056
b_2	6.71E-03	−1.27E-02
b_3	8.31E-05	−1.18E-03
b_4	5.28E-06	1.39E-04
R^2	0.72	0.70

photosynthesis (Jordan & Ogren 1984; Long 1991). This low response was found in early spring when the response of the midday CCER to CO_2 concentration was only 1.19. For late spring this increased to 1.53.

Midday CCER and evapotranspiration differences in early spring between the CO_2 treatments (Figs 4 and 5) can have two causes: LAI and/or activity per unit leaf area. Based on the experiments in the OTCs, a difference in leaf area or biomass in early spring is unlikely (Fig. 1, Table 1). So the activity per unit leaf area must have been increased in response to CO_2 concentration. Similarly, the reduction of evapotranspiration rates during the early spring indicated that elevated CO_2 did have an effect through specific activity and not LAI.

Only after temperatures and irradiance levels increased, did an increase in the CO_2 response ratio take place (Fig. 7). In contrast to winter wheat, spring wheat (Mulholland *et al.* 1997) and nonvernalized winter wheat (Du Cloux *et al.* 1987) grown at a continuous 20 °C, showed differences in LAI right from the start of the experiment.

The evapotranspiration rate was lower for the elevated CO_2 treatment, as has been shown in many instances before (Eamus 1991). This contrasted with findings for spring wheat and faba bean, where evapotranspiration per unit ground area was unaffected by CO_2 treatment during the greater part of the season (Dijkstra *et al.* 1993).

Temperature–irradiance interaction

Low irradiance and low temperature were the cause for the low response of CCER to elevated CO_2 during early spring. However, a significant contribution of the interaction between irradiance and temperature was also found (Table 5). The increase in the CO_2 response of midday CCER, going from early to late spring, can be explained in terms of the effects of irradiance, temperature and the interaction of irradiance and temperature. The CO_2 response ratio of midday CCER at the averaged

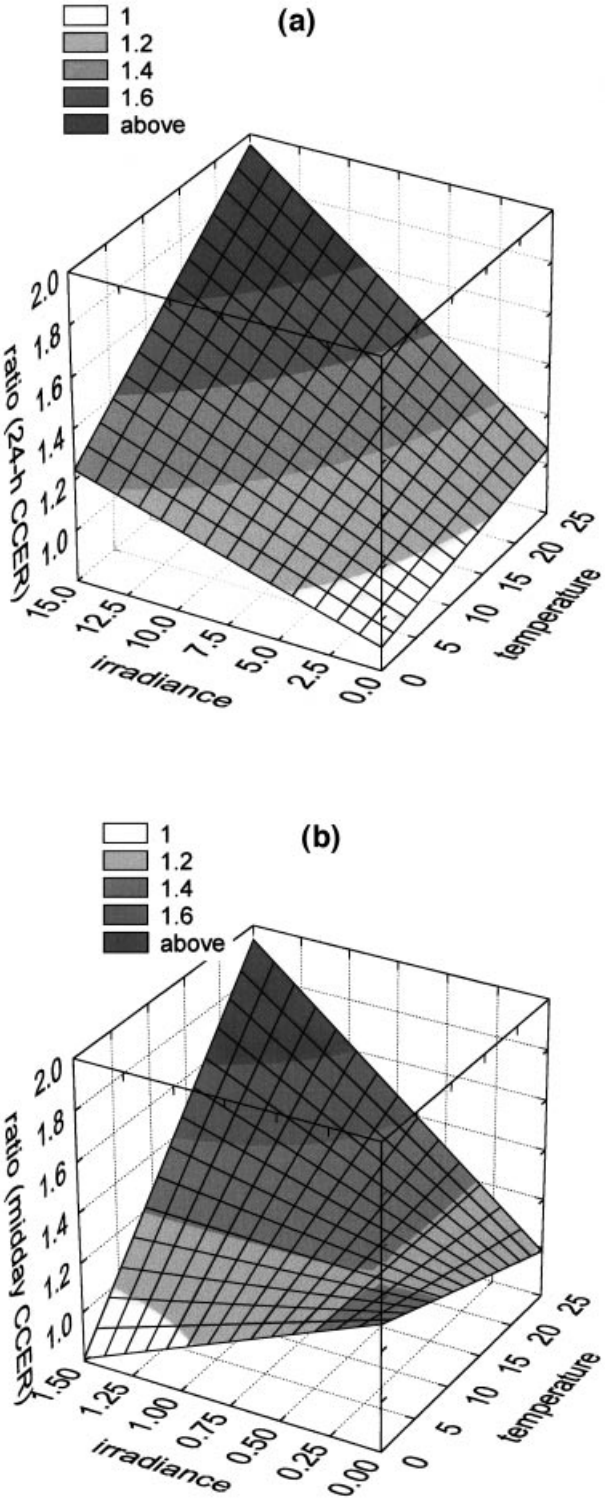

Fig. 6 Regression surface of the CO_2 response ratio of 24-h CCER against the 24-h mean temperature (°C) and irradiance (MJ m^{-2} d^{-1}) (a) and the CO_2 response ratio of the midday CCER against midday temperature (°C) and irradiance (MJ m^{-2} h^{-1}) for the period from 50 to 150 days. Regression was a multiple regression of temperature, irradiance level and their interaction.

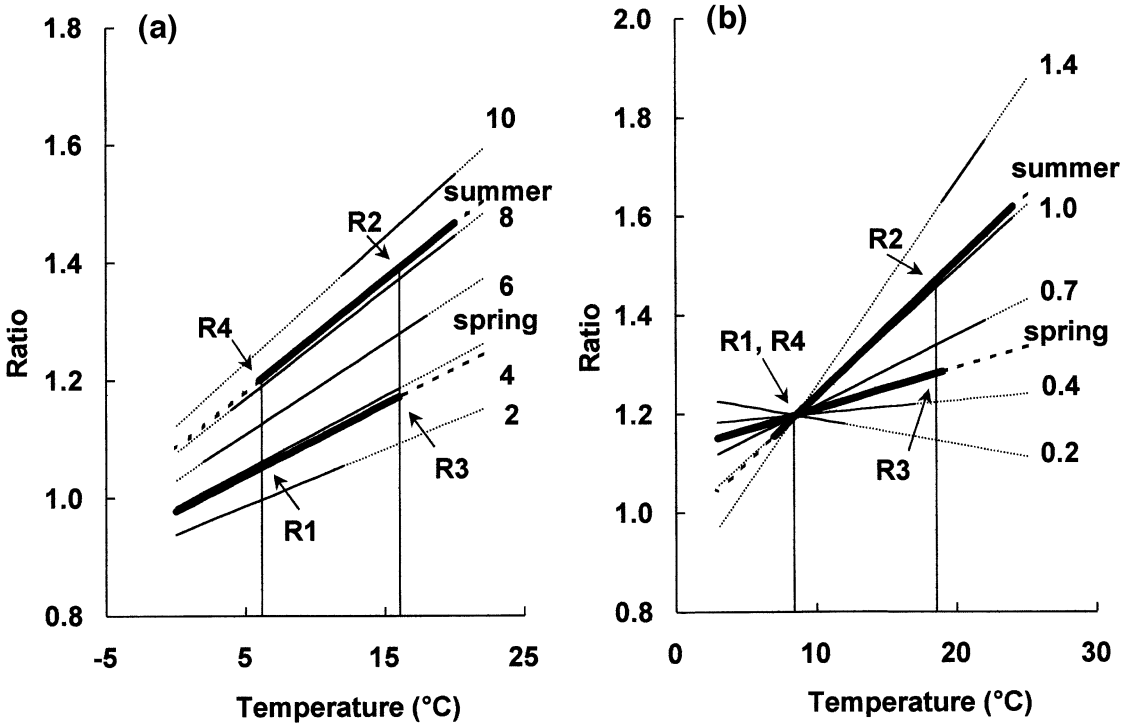

Fig. 7 CO_2 response ratio of 24-h CCER against the 24-h averaged temperature for different irradiance levels (numbers next to lines are MJ m^{-2} d^{-1}) (A) and response ratio of midday CCER against the midday temperature for different irradiance levels (numbers next to the lines are MJ m^{-2} h^{-1}). Individual regression lines were calculated from Table 6. Broken lines indicate temperature and irradiance conditions not encountered in the data-set. Bold lines indicate conditions resembling averaged early and late spring irradiance intensities. R1 and R2 refer to, respectively, averaged early and late spring, R3 to early spring conditions with late spring temperatures, and R4 to early spring conditions with late spring irradiance level.

temperature and irradiance level in the early spring (R1 in Fig. 7b, Table 6) is lower than the ratio at averaged temperature and irradiance level in the late spring (R2 in Fig. 7b). Changing the temperature from early spring values to late spring values, without changing the irradiance level (going from R1 to R3 in Fig. 7b), increased the CO_2 responsiveness (Table 6). Increasing the irradiance level to late spring values did not have any effect for the responsiveness to CO_2 (R1 to R4 in Fig. 7b). It is concluded that of the total increase in the response of midday CCER to CO_2 concentration, about 31% is caused by the increase in temperature, while 73% is explained by the interaction between irradiance and temperature. Similarly it was shown that the temperature difference explained 35% of the increase in the CO_2 response ratio of the 24-h CCER, going from early to late spring. Irradiance explained 44% of the increase in the response, while the interaction between irradiance and temperature only explained 21% (Fig. 7a, Table 6).

Below a temperature of 19 °C (Idso *et al.* 1987; Kimball *et al.* 1993) no stimulation of growth to CO_2 doubling, or even a negative effect was found. The negative response of *Lolium perenne* shoot biomass to CO_2 concentration during the winter was already mentioned (Nijs & Impens 1996). The results from the regression analysis indicated that such a negative effect would be possible for midday-CCER under low temperature and high irradiance levels (Fig. 7b). However, these conditions were not encountered during the experimental period, so no proof of this hypothesis can be deduced from this study. The 24-h CCER did not indicate these possible negative effects of enhanced CO_2 concentrations by low temperature and high irradiance sums (Fig. 7a).

Our results have a number of similarities with the modelling results of Long (1991): under low temperatures, the calculated response of light-saturated leaf photosynthesis was around 4%, very close to our 5% during the early spring period when LAI was low. Maximum values of the CO_2 response of the 24-h CCER increased up to 75% at the highest irradiance level (Fig. 7a) and 53% averaged over the late spring, compared to about 50% stimulation at a temperature of 25 °C and irradiance of 36 mol m^{-2} d^{-1} (fig. 8 in Long 1991).

Long (1991) explained the difference in the response of canopy photosynthesis between a tundra vegetation and a salt marsh vegetation by a difference in temperature,

Table 6 Temperature, irradiance levels, observed and estimated response ratio of CCER to CO_2 concentration averaged over the early and late spring. Estimated CO_2 response ratio is based on combinations of midday and 24-h temperature and irradiance values, using the regression equations from Table 5. % Explained is the difference in response ratio between the calculated value and the early spring value as a percentage of the total difference between the late and early spring values.

	24-h				Midday			
	Temp (°C)	Radiation (MJ m^{-2} d^{-1})	Response ratio (–)	% explained	Temp (°C)	Irradiance (W m^{-2})	Response ratio (–)	% explained
Early spring observed	6.2	3.70	1.05		8.4	0.551	1.19	
Late spring observed	16.0	8.41	1.40		18.6	1.037	1.53	
Early spring calculated	6.2	3.70	1.05		8.4	0.551	1.20	
Early spring plus T_{late}	16.0	3.70	1.17	35	18.6	0.551	1.28	31
Early spring plus Q_{late}	6.2	8.41	1.20	44	8.4	1.037	1.19	–4
Late spring calculated	16.0	8.41	1.39		18.6	1.037	1.47	

with light being of no additional importance. Simulation studies by Grashoff *et al.* (1996) for spring wheat and faba bean crops similarly showed that temperature was more important than light intensity in explaining variability in the response to CO_2 concentration. However, from the present study it became clear that irradiance and temperature did have strong interactions on the response of canopy photosynthesis of winter wheat to CO_2. This interaction was also found when the late spring period was analysed separately (results not shown).

We conclude that in order to understand the variability in the response to CO_2 concentration (by species, year-to year or over the season), variation in microclimatological parameters can play a dominant role.

Concluding remarks

Under low temperature and low irradiance levels, experienced in early spring, the CO_2 effect on winter wheat growth and canopy gas-exchange is absent. This agrees with knowledge on the effect of temperature on photorespiration. Then, with increasing temperatures and/or irradiance levels towards the end of early spring, CO_2 begins to have an effect: slightly increased canopy photosynthesis and decreased evapotranspiration rates at the highest temperatures and irradiance levels (midday). As a consequence, N concentration per unit dry weight and ground area decrease and nonstructural carbohydrates increase. However, plant growth itself is not responding, due to temperature-restrictions on sink activity. Only when the temperature rises further still, does growth respond, with root growth the first to increase. This enhanced root growth accelerates the N-uptake so that at the end of the experiment, elevated CO_2 has a higher total amount of N per unit ground area. The period where CO_2 concentration begins to have an effect, but growth is still temperature controlled, may be a period where acclimation of leaf photosynthesis is expected (Drake *et al.* 1997).

The alternative explanation, that the response to CO_2 concentration varies with developmental stage, cannot be completely ruled out. However, since even during late spring, a relation between temperature and irradiance with the CO_2 responsiveness of CCER was found, this seems less likely to be of overriding importance. However, effects of CO2 on leaf area senescence may have occurred during the summer.

The outcome of this study confirmed the notion that species growing early in the season are expected to benefit less from CO_2 doubling than species that grow later and under higher temperatures (Dijkstra *et al.* 1996).

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